AN INVESTIGATION OF THE ROLE OF JUVENILE HORMONE IN DEALATION AND FLIGHT IN *Solenopsis invicta* FEMALE ALATES

By

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For

Susie E. Burns,
Roland H. Burns,
Joan M. Burns, and
Loretta S. Burns

Thank you for always being there.
I love you *so* much.

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Abstract of Dissertation Presented to the Graduate School
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AN INVESTIGATION OF THE ROLE OF JUVENILE HORMONE
IN DEALATION AND FLIGHT IN *Solenopsis invicta*
FEMALE ALATES

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Solenopsis invicta female alates normally dealate following a mating flight. However, female alates can shed their wings within the nest when the queen is absent. Results from this investigation revealed that the time at which alates dealate in the presence of workers and brood is not dependent upon sexual maturity. One hundred percent dealation occurred within 108h for newly-eclosed, 7-day-old, and 14-day-old alates. However, the exclusion of workers and brood increased the time required for 100% dealation, which occurred within 156h for alates in isolation and those in groups.

The role of juvenile hormone (JH) in dealation was examined with the use of topical applications of JH III and precocene II, a JH antagonist, on female alates. Juvenile hormone III treatments, ranging from 0.01-20ng, induced dealation of female alates in the

presence of the queen. One hundred percent dealation occurred within 10h of applying 20ng JH III compared with 108h for 0.01ng JH III. Topical precocene II treatments of 90 and 100ug inhibited 83 and 97% of alates from shedding their wings during a 108-h period in queenless colonies, respectively; however, 10 and 20ng JH III applications stimulated 95 and 100% precocene-treated alates to dealate within 48h, respectively.

The sizes of the corpora allata (CA) were measured in female alates and found not to change significantly upon reaching sexual maturity or when alates were treated with 1ng JH III. The sizes of the CA were reduced significantly in alates topically administered 100ug precocene II.

The role of JH in flight of female alates was examined with topical applications of precocene II. Alates topically treated with 100ug precocene II did not exhibit pre-flight behaviors associated with a mating flight, and once tethered, these alates could not be induced to fly.

Pre-copulatory behaviors associated with a mating flight do not contribute significantly to dealation in female alates. Some alates that exhibited pre-copulatory behaviors took from 132-168h to shed their wings.

The identification and quantitation of JH compounds in hemolymph of female alates were determined with gas

chromatography-mass spectroscopy using chemical ionization. Juvenile hormone III was the only JH homolog detected, and the average amount of JH per microliter of hemolymph was 0.29±0.13pmol.

CHAPTER I

REVIEW OF LITERATURE

Introduction

Fire ants belong to the genus *Solenopsis* (Hymenoptera: Formicidae: Myrmicinae), and North America is home to four native fire ant species and two introduced species from South America. The native species are *S. amblychila* Wheeler, *S. aurea* Wheeler, *S. geminata* (Fabricius), and *S. xyloni* (MacCook); the two introduced species are *S. invicta* Buren and *S. richteri* Forel. *Solenopsis invicta*, the red imported fire ant, is of great concern because of its agricultural and medical impact in the United States (Adams and Lofgren, 1981; Trager, 1991).

The geographic distribution of *S. invicta* in South America includes Argentina, Bolivia, Brazil, Paraguay, and Uruguay, (Trager, 1991). *Solenopsis invicta* was unintentionally introduced into the United States, possibly in the 1930s. These fire ants were probably introduced with cargo or in soil used as ballast in ships arriving from South America and unloaded at the port of Mobile, Alabama (Buren, 1972; Buren et al., 1974). *Solenopsis invicta* is well-adapted for the invasion of disturbed areas created by humans through the clearing

of land for agricultural purposes, housing, roads, etc. (Tschinkel, 1993). Although the spread of *S. invicta* is hindered by long periods of dry or cold conditions (Morrill, 1977), the fire ant is presently found in Puerto Rico and fourteen U. S. states: Alabama, Arkansas, Florida, Georgia, Louisiana, Mississippi, North Carolina, South Carolina, Tennessee, Texas, Virginia, (Stimac and Alves, 1994), California, New Mexico (Weaver-Missick, 1999), and Oklahoma (Vinson and Sorensen, 1986). The rapid expansion of the fire ant in the United States may be attributed to transport by human beings via car, rail, or air (Lofgren, 1986), and to a lesser degree, to dispersal through mating flights (Markin et al., 1971). Lower population levels of *S. invicta* are found in its South American homeland than in North America (Porter et al., 1992), and it has been suggested that the fire ant has expanded in the United States because of the relative absence of predators, parasites, pathogens, and ant competitors--factors which maintain low ant populations in the fire ant's native homeland (Buren et al., 1978; Porter et al., 1992).

Solenopsis invicta's impact on man is well-known. Fire ant workers inject venom--a series of *trans*-2-methyl-6-alkyl or alkenyl piperidines (Vander Meer and Morel, 1995)--into their victims,

resulting in a burning sensation (MacConnell et al., 1970). The areas of infection become red and swell into bumps. Pustules form within a day (Vinson and Sorensen, 1986, 1999). It has been estimated that up to 14 million people may be stung annually (Adams, 1986), and in some individuals, serious allergic reactions may occur (Rhoades, 1977; Paull, 1984).

The red imported fire ant is an opportunistic feeder and is considered an agricultural pest. The fire ant causes serious damage to corn and soybeans by feeding on germinating seeds. It also feeds on buds and developing fruit of berries, beans, citrus, and okra (Adams et al., 1983). The fire ant also causes extensive damage by girdling young trees, in particular, citrus and pecan (Vinson and Sorensen, 1986). Losses due to crop damage from the ant is assessed to approach 40 to 50 million dollars a year (Stimac and Alves, 1994).

Solenopsis invicta has a major impact on wildlife. Several ant species native to North America have been negatively affected by the fire ant's presence. For example, both *S. invicta* and *S. xyloni*, a closely-related native species, prefer open environments over woodlands, but *S. xyloni* has completely vanished in areas occupied by *S. invicta*. A second native species, *S. geminata,* favors both open and wooded areas, but the ant has been restricted to less

disturbed territories within the range of *S. invicta* (Wilson and Brown, 1958). *Solenopsis invicta*, as a predator/scavenger, directly threatens the survival of ground-nesting and ground-inhabiting animal species, both invertebrates and vertebrates. For example, red imported fire ants often nest in burrows containing eggs of gopher tortoises, *Gopherus polyphemus*. The eggs and newly-hatched young of this endangered species are vulnerable to predation from *S. invicta* (Allen et al., 1994). The fire ants may indirectly affect animal abundance and diversity by reducing food supply, thereby altering the food web in many ecosystems (Vinson and Sorensen, 1986).

Though *S. invicta* is mainly characterized as a pest, it does have some beneficial attributes. By its aggressive feeding on other insects, the fire ant has benefited a number of crops such as sugarcane, corn, and cotton by controlling the sugarcane borer (Long et al., 1958), the corn earworm (Vinson and Sorensen, 1986), and the boll weevil (Jones and Sterling, 1979). However, the fire ant's omnivorous eating behavior has also reduced the number of beneficial insects such as ladybird beetle larvae (Vinson and Sorensen, 1986).

Some of the methods currently being used to control *S. invicta* are mound drenches, surface dusts, injected toxicants, fumigants,

baits, and biological control agents (Vinson and Sorensen, 1986; Stimac and Alves, 1994). State and federal agencies have spent more than 200 million dollars in the control of *S. invicta*; however, these efforts have done very little to halt the spread of the fire ant, and many control methods have only hastened the fire ant's invasion by eliminating competing native ant species (Diffie and Sheppard, 1990). Despite the many attempts to reduce the number of mounds in the United States, *S. invicta* continues to extend its range.

Colony Foundation

During the spring, summer, and fall seasons, a mature *S. invicta* colony actively produces reproductives (alates) (Vargo and Fletcher, 1987). While in the nest, female alates are prevented from shedding their wings or developing their ovaries by a primer pheromone produced by the functional queen (Fletcher and Blum, 1981a,b, 1983a). Male and female alates leave the parental nest when sexual maturity has been reached and when suitable weather conditions stimulate them to engage in a mating flight (Markin et al., 1973).

Mating flights usually take place in early afternoon, one or two days following rain, with temperatures ranging between 20° and

32°C, with a minimum of 80% humidity, and wind gusts that usually do not exceed 5mph (Rhoades and Davis, 1967; Markin et al., 1971; Milio et al., 1988). Prior to a flight, workers create exit holes on the surface of the mound and exhibit an alarm-like behavior over the mound (Markin et al., 1971; Obin and Vander Meer, 1994). This behavior is induced by mandibular gland secretions from the male and female alates (Alonso and Vander Meer, 1997). Usually males commence flying before female alates. Males leave the mound first, climb onto nearby vegetation, and eventually fly off. Afterwards, female alates emerge and ascend into the swarm of males in the air. A female alate will obtain a lifetime supply of sperm from a single male during this flight (Page, 1986). Males die after mating, while inseminated females continue flying in search of reflective or moist areas on which to land and establish a colony (Markin et al., 1973; Vinson and Sorensen, 1986).

Once an inseminated queen lands in a suitable area, she breaks off her wings (dealates) with her middle and hind legs. The wing muscles then begin to degenerate (Markin et al., 1973). The amino acids and peptides from wing muscles are released into the hemolymph and are used for egg production and as food for the first batch of larvae (Toom et al., 1976).

Following dealation, the queen digs into the ground and makes a nuptial chamber, and within 24h, the queen begins laying her first clutch of eggs (Toom et al., 1976). This early period of colony growth is the founding stage (Hölldobler and Wilson, 1990).

The first workers to eclose are referred to as nanitics or minims and are the smallest workers produced by the colony (Porter and Tschinkel, 1986). Once eclosed, nanitics immediately begin constructing the new nest, tending the queen and secondary brood, and foraging for food (Oster and Wilson, 1978). Nanitics do not undergo age polyethism as mature colony workers do (Mirenda and Vinson, 1981). The distinctive size, behavior, and venom alkaloid patterns of nanitics compared to other workers suggest that nanitics are of a separate caste (Vander Meer, 1986). Within a month, worker size distribution changes, and the colony begins to grow. Within six months, the mound has thousands of workers and is visible in the field (Oster and Wilson, 1978). The mature colony consists of many minor workers, some media workers, and a few major workers (Wilson, 1978). This period of relatively rapid colony growth, consisting of the production of many workers, is considered the ergonomic stage (Hölldobler and Wilson, 1990).

The accelerated growth of the colony is aided by worker division of labor based on age polyethism and physical size.

Young workers tend to the queen and brood, and older workers act as reserves. Reserves also tend to brood, maintain and defend the colony, and retrieve food discovered by the oldest workers, foragers (Mirenda and Vinson, 1981). Along with age, the size of the worker may determine its duties. Nurses are principally young minor workers, and a smaller number are media workers. Major workers do not tend brood but move to the periphery of the nest with reserves (Glancey et al., 1973).

A mature colony has reached the reproductive stage when winged females and males (reproductives) are produced. Reproductives will eventually engage in a mating flight, and the cycle will repeat itself (Hölldobler and Wilson, 1990).

Releaser Pheromones in *Solenopsis invicta*

Chemical signals pervade nearly all insect activities (Birch and Haynes, 1982). Chemicals used in communication are known as semiochemicals. Semiochemicals are further classified as either allelochemicals or pheromones. Allelochemicals mediate interactions among individuals of different species, while pheromones are used to communicate among individuals of the same species (Howse et al., 1998). Pheromones are described as either releasers (chemicals that induce immediate behavioral

responses) or primers (chemicals that affect the physiological state of the insect but do not cause an immediate behavioral response) (Evans, 1984). A chemical signal can be multicomponent and can be comprised of a blend of chemicals from more than one gland (Hölldobler, 1995). The average worker ant has been described as "a walking battery of exocrine glands," developed to a degree far beyond that of nonsocial hymenopterans. Many of these glands have been identified in the production of semiochemicals (Hölldobler and Wilson, 1990).

For example, in *S. invicta,* the Dufour's gland is the source of trail pheromones. Once a worker has located a food source, she lays a chemical trail with glandular secretions to aid nestmates to food. Compounds identified as trail pheromones are (Z,E)-α-farnesene, (E,E)-α-farnesene (Vander Meer, et al., 1981, 1988), (Z,E)-homofarnesene, and (E,E)-homofarnesene (Alvarez et al., 1987).

Solenopsis invicta colony members display heightened excitement and aggression behaviors prior to a mating flight. Obin and Vander Meer (1994) reported that they were able to stimulate alates to fly in the laboratory, and they demonstrated that chemical signals from both female and male alates attracted workers, promoted alarm-recruitment behaviors in workers, and

encouraged alate retrieval by workers. Alonso and Vander Meer (1997) found that the mandibular gland was the source of these excitant pheromones.

The *S. invicta* queen elicits tending behaviors from workers (Hölldobler and Wilson, 1990). With the use of surrogate queen and olfactometer bioassays, Vander Meer and his colleagues (1980) demonstrated that *S. invicta* worker attraction and/or aggregation is elicited by compounds produced from the poison sac of the queen. Rocca et al. (1983a,b) subsequently isolated and identified the pyrone (E)-6-(1-pentenyl)-2H-pyran-2-one, invictolide, and dihydroactinidiolide from whole queen extracts. Surrogate queen bioassays indicated that the pyrone and invictolide elicited behavioral responses by workers; however, dihydroactinidiolide was found to be inactive (Glancey et al., 1984). Queen pheromones responsible for such releaser effects are synthesized in detectable amounts in about two days following dealation (Vargo, 1999). *Solenopsis invicta* eggs are marked with queen pheromones and with antimicrobial alkaloids. The sting of the queen is used in the egg-laying process, and poison sac contents are placed on eggs as they are laid. Secretions from the poison sac protect eggs from microorganisms. In addition, the deposition of the queen-attraction pheromones on the eggs induce worker attraction toward eggs

(Vander Meer and Morel, 1995), and the release of these pheromones is most likely correlated with egg-laying rates (Fletcher and Blum, 1983a,b). Queen pheromones and the antimicrobial products that are laid on the eggs give them additional protection, thereby increasing egg survivorship (Vander Meer and Morel, 1995).

Queen Primer Pheromones in *Solenopsis invicta*

To date, no ant primer pheromones have been identified, and very little detailed information exists about them. The major difficulty in investigating primer pheromones is the need for more sensitive, reliable bioassays. Unlike the relatively swift behavioral responses observed in releaser pheromones, the subtle physiological effects of primer pheromones may not be apparent for days or even weeks (Vargo, 1998). Queen primer pheromones appear to control such basic processes as caste determination and reproductive development in ant colonies (see Wilson, 1971 and Hölldobler and Wilson, 1990 for reviews).

Regulation in the Production of Sexuals. Vargo and Fletcher (1987) found that *S. invicta* polygyne colonies, under both field and laboratory conditions, produce fewer numbers of female and male sexuals than monogyne colonies during spring, summer, and fall—seasons when sexuals are actively produced. And based on these

findings, it was suggested that a negative correlation exists between queen number and the production of sexuals.

Subsequent experiments conducted by Vargo (1988) showed that workers are required to make contact with the queen(s) to acquire queen chemicals so that they are distributed throughout the colony to inhibit the production of sexuals. This discovery was made by placing queen corpses in small cages that prevented direct contact with workers but allowed volatile chemicals to pass.

Vargo (1988) suggested that queen primer pheromones function indirectly in controlling the number of sexuals in the colony by causing workers to limit the quantity and/or quality of food to female larvae, thereby producing workers; in contrast, male larvae are killed by workers. In addition, Vargo and Fletcher (1986) reported that another form of pheromonal control involves workers executing both female and male late instars after they have been sexualized.

Social control over reproduction is one component of insect societies (Oster and Wilson, 1978). The use of queen primer pheromones in directing workers in their attention to the survival of sexuals may be one means by which the functional queen limits the number of potential egg-laying competitors within the colony.

Regulation of Queen Number. The reduction of queen number to a single egg-laying queen in *S. invicta* monogyne colonies is usually accomplished with the execution of supernumery queens by workers. For example, newly-mated queens often unite in groups and participate in the rearing of first brood; however, workers that have emerged execute all but one queen, creating a monogyne colony (Tschinkel and Howard, 1983). Vander Meer and Alonso (in press) discovered that queenless monogyne workers readily accepted newly-mated queens; however, the survival of queens declined over time. Fletcher and Blum (1981a, 1983b) hypothesized that the quantity of pheromone from an individual queen correlates with fecundity and that workers will only tolerate a certain level of these queen substances in the colony. Hence, when the queen pheromone exceeds a particular threshold, workers will execute queens that are least fecund first, thereby retaining the most reproductively active queens.

Control Over Oviposition in Functional Queens. Individual functional queens in *S. invicta* polygyne colonies are less physogastric and lay fewer eggs than queens in monogyne colonies (Fletcher et al., 1980). Queen number is negatively correlated with the number of eggs laid per queen (Greenberg et al., 1985). Vargo (1992) demonstrated that queens from polygyne colonies mutually

inhibit fecundity in one another, possibly by reducing juvenile hormone titers. Vargo found that the introduction of queen corpses into small colony fragments consisting of a single queen, workers, and brood suppressed fecundity of test queens compared to control queens receiving corpses of alates. In addition, topically applying methoprene, a juvenile hormone analogue, onto individual live queens increased fecundity, suggesting that ovary development in queens is controlled by endogenous levels of juvenile hormone.

Control Over Dealation and Oviposition in Female Alates. Mature *S. invicta* colonies actively produce female and male sexuals throughout the year, except during the winter months (Vargo and Fletcher, 1987). Sexuals remain in the nest until suitable weather conditions induce them to engage in a mating flight (Lofgren et al., 1975). While female alates are in the nest, dealation, wing muscle histolysis, and oviposition are inhibited by a nonvolatile primer pheromone released from the egg-laying queen(s) (Fletcher and Blum, 1981a,b, 1983a). Detectable levels of the primer pheromone responsible for inhibiting dealation are present in uninseminated sexuals approximately three days following wing casting (Vargo, 1999).

The dealation inhibitory pheromone is very effective. Mature monogyne colonies may consist of about 230,000 workers, tens of

thousands of immatures, and 5,000 virgin queens, all dispersed over about 40 liters of nest (Markin et al., 1973). Yet, the queen primer pheromone prevents female sexuals from dealating, a behavior that normally precedes wing muscle histolysis and oviposition (Fletcher and Blum, 1983b).

Juvenile Hormone in the Control of Dealation and Flight in *Solenopsis invicta* Female Sexuals

The corpora allata (CA) are endocrine glands located behind the brain of an insect. The CA produce juvenile hormone (JH), which regulates metamorphosis and reproduction (Evans, 1984). Precocenes, chromene derivatives isolated from plants of the genus *Ageratum*, have been shown to cause atrophy of the CA (Tobe and Stay, 1986). The JH-antagonistic effects of precocene have been studied in some insects to evaluate the possible role of JH in different physiological and behavioral events (Bowers, et al., 1976; Goewie et al., 1978, cited in Bowers, 1983; Rankin and Riddiford, 1978, 1980; Müller et al., 1979; Rembold et al., 1979; Bowers, 1983). However, as yet, precocene has not been incorporated into any studies with *S. invicta* to help assess how JH functions within the body of the ant and how the hormone may ultimately affect certain behaviors or developmental events. In *S. invicta*, the wings

of female alates are shed following a mating flight or the removal of the functional queen(s) from the colony, and it has been proposed that JH plays a critical role in stimulating alates to remove their wings (Kearney, 1977; Barker, 1979; Fletcher and Blum, 1981a,b, 1983a,b; Vargo and Laurel, 1994). Results from experiments involving the effects of topical JH and precocene treatments on dealation in female alates may provide further evidence of the role of JH in wing casting.

While in the colony, *S. invicta* female alates are prevented from shedding their wings by the queen dealation inhibitory primer pheromone; however, once pheromonal influences have been removed, these alates shed their wings within three days (Fletcher et al., 1983a,b). Results from previous studies including topical treatments of synthetic JH suggest that JH may be the physiological inducer for dealation in *S. invicta* female alates (Kearney et al., 1977; Barker, 1979). Vargo and Laurel (1994) noted that methoprene-treated alates even shed their wings while in the presence of the functional queen. These researchers hypothesized that the dealation inhibitory primer pheromone prevents the CA of alates from producing high JH titers, thereby hindering dealation.

Although hormone levels have not been systematically measured as a function of aging in insects (Lockshin and

Zimmerman, 1983), it has been reported that in some insects, JH levels increase as adults mature (Kramer, 1978; Huibregtse-Minderhoud et al., 1980; Huang et al., 1994). For example, in the European honey bee, *Apis mellifera*, older adult workers in queenright colonies produce higher concentrations of JH than younger individuals (Rutz et al., 1976; Fluri et al., 1982), and topical or oral treatments of JH analogues--hydroprene or methoprene--induce precocious foraging, which is an age-related behavior (Robinson, 1985; Robinson and Ratnieks, 1987). Though not investigated, it may be possible that even under pheromonal control, adult *S. invicta* female alates of different ages secrete different amounts of JH; hence, once these female sexuals are liberated from functional queen influences, the time at which alates are induced to shed their wing is in accordance with age-related JH titers. In addition, if JH levels must reach a threshold in order for dealation to occur, as has been suggested (Fletcher and Blum, 1983a,b), it is of special interest whether the CA of sexually immature (newly-emerged) alates become fully functional once released from pheromonal inhibition, thereby producing sufficient JH levels to stimulate dealation.

Once sexual maturity has been reached and optimal environmental conditions exist, *S. invicta* reproductives engage in a

mating flight (Markin et al., 1973). It is unknown whether or not JH plays a role in stimulating them to fly, a behavior that precedes mating. Robinson and Ratnieks (1987) demonstrated that topically or orally treating *A. mellifera* workers with JH analogs induced premature foraging in young bees, individuals that would have normally remained in the nest. Furthermore, Rankin and Rankin (1980) reported that topical JH treatments on female and male ladybird beetles, *Hippodamia convergens*, significantly increased flight duration. These and other examples (see Goldsworthy and Wheeler for review) suggest that JH may be important in flight in *S. invicta* females.

The surgical removal of the CA (allatectomy) and the subsequent administration of JH or analogues can provide more definitive data as to the role of JH in insects. However, because of the intricate manual operations needed to perform allatectomy, precocene has been used as a chemical means of inhibiting JH biosynthesis in some insects (Bowers, et al., 1976; Goewie et al., 1978, cited in Bowers, 1983; Rankin and Riddiford, 1978, 1980; Müller et al., 1979; Rembold et al., 1979; Bowers, 1983). Precocenes affect physiological and behavioral events in some insects. For example, Müller and his colleagues (1979) reported that the CA of milkweed bugs, *Oncopeltus fasciatus,* incubated *in*

vitro in medium consisting of 1ug/ml of precocene II no longer secreted JH when re-implanted into fifth (last) instars. Bowers et al. (1976) reported that precocene I and II induced precocious metamorphosis in *O. fasciatus*. The nymphs molted to seemingly normal third and fourth instars but prematurely molted to tiny adults, thereby excluding the fifth instar. Studies incorporating exogenous JH and precocene treatments on *O. fasciatus* (Rankin and Riddiford, 1978) and the convergent ladybird beetle, *Hippodaemia convergens* (Rankin and Rankin, 1980), suggest that JH is the physiological inducer of flight behavior that aids dispersal of these adult insects.

There are, however, conflicting data regarding the effects of precocene II in the honey bee. Rembold et al. (1979) were unable to demonstrate that precocene has an anti-JH effect in *A. mellifera* development, while results from Goewie et al. (1978, cited in Bowers, 1983) showed that the treatment of honey bee queen larvae with precocene II caused the formation of worker-like intermediates, which is in accordance with the expected role of JH in regulating the development of queens. Goewie and his associates also reported that precocene caused atrophy of the CA.

The effects of precocene have not yet been investigated in *S. invicta*, but studying these effects may be invaluable in understanding fire ant endocrinology. Precocene and JH treatments

may be administered to *S. invicta* alates to help determine whether JH is the physiological inducer in both flight and wing casting.

Fletcher and Blum (1981a,b) reported that fire ant alates are induced to shed their wings within two days of the removal of the functional queen. However, newly-mated queens dealate within 4h after descending from a mating flight (Markin et al., 1972), an event that may last about 20min (Milio et al., 1988). Kearney (1977) reported that alates topically treated with 1ng of synthetic JH were stimulated to dealate within 6-8h, whereas longer time periods were observed with smaller doses. The results from this study suggest the possibility that newly-mated queens are induced to shed their wings at a faster rate than unmated females because of increased levels of JH present in their bodies during the course of mating flight activities.

Individual behaviors associated with the mating flight have yet to be investigated to determine whether one or a combination hastens dealation, possibly by increasing JH titers. Specific behaviors have been reported to regulate JH levels in some insects. For example, Huang and Robinson (1992) proposed that the interaction of honey bee workers of different ages influences JH titers and behavioral development. Under normal circumstances, nurses produce lower JH titers than older workers, foragers

(Robinson, 1987). However, Huang and Robinson (1992) found that when nurses are isolated from foragers, nurses produce unnaturally high JH titers and engage in foraging activities. It was hypothesized that under natural conditions, foragers suppress JH production in young workers, either by chemical or physical means, and as foragers die, younger workers receive less inhibition, resulting in behaviors associated with older workers (Huang and Robinson, 1992).

Mating is another potential factor encouraging dealation in inseminated *S. invicta* female alates. In some insect species, mating is responsible for activating and enhancing egg development, and in some examples, these effects are credited to an increase in JH (see Davey, 1983 for review). In *Diploptera punctata*, for example, mating stimulates the CA through signals transmitted by nerve connections (Engelmann, 1959; Stay and Tobe, 1977). In addition, mating may involve the direct transfer of JH from the male to the female. *Heliothis virescens* males are known to transfer JH to females, and allatotropic stimuli in the female may accompany this transfer to enhance egg production (Park et al., 1998).

Based on the results from a limited number of studies regarding the use of synthetic JH applications on *S. invicta* female alates, JH appears to play a critical role in dealation in female

sexuals (Kearney et al., 1977; Barker, 1979; Vargo and Laurel, 1994). The incorporation of precocene treatments with JH applications may provide further evidence of JH's role in wing casting and in flight. In addition, an examination of alate activities associated with a mating flight may provide information about the behavior(s) critical to post-mating dealation.

Research Aims

There is very little information in the literature regarding the role of the endocrine system in dealation and in flight of *S. invicta* female sexuals. Results from studies involving the use of topical treatments of JH or JH analogues on *S. invicta* female alates suggest that JH is the natural stimulant to wing casting. The incorporation of both JH and precocene applications on female alates may provide further evidence of JH as the natural inducer to dealation, as well as to flight. In addition, the identification of the natural JH compound(s) in female alates will provide valuable information to the study of fire ant endocrinology.

The present study will investigate the role of JH in dealation and in flight of *S. invicta* female sexuals. The activities associated with the mating flight will be examined to identify pre-copulatory behaviors potentially crucial in dealation of newly-mated female

sexuals. In addition, the hemolymph of female alates will be analyzed for the identification and quantitation of JH. In order to accomplish these objectives, the following studies will be conducted: 1) a comparison of the rates of dealation of *S. invicta* female alates of different ages and a comparison of the CA sizes of sexually immature and mature female alates; 2) an investigation of the role of the CA in dealation, using topical JH and precocene treatments, and a comparison of the CA sizes of JH and precocene-treated female alates; 3) an investigation of the role of the CA in inducing alates to fly, using topical precocene and JH treatments; 4) an examination of activities associated with a nuptial flight to determine potential behaviors influencing dealation of newly-mated queens; 5) the identification and quantitation of naturally-occurring JH compounds in the hemolymph of female alates, with gas chromatography-mass spectroscopy using chemical ionization.

CHAPTER II

DEALATION RATES OF *Solenopsis invicta* FEMALE ALATES OF DIFFERENT AGES

Introduction

Sexual forms of *Solenopsis invicta* are actively produced during the spring, summer, and fall seasons (Vargo and Fletcher, 1987). Both female and male reproductives reach sexual maturity about 7-10 days after eclosing from the pupal stage (Glancey, unpublished data, cited in Lofgren et al., 1975). They then remain in their parental nest and do not engage in a mating flight until suitable weather conditions prevail. Mating flights usually take place in the early afternoon, one or two days following rain. Temperatures on the day of the flight usually range between 20° and 32°C, with a minimum of 80% humidity, and wind gusts that usually do not exceed 5mph (Rhoades and Davis, 1967; Markin et al., 1971; Milio et al., 1988). Following insemination, females fly to moist or reflective surroundings on which they land and establish a colony (Markin et al., 1978; Vinson and Sorensen, 1986). Dealation takes place within 4h after the flight (Markin et al., 1972). Flight muscles degenerate, and amino acids and proteins provided by the muscles contribute to egg production (Vinson, 1986).

Once the colony is established, the functional queen exerts control over female alate nestmates with a primer pheromone that suppresses wing casting and ovary development. However, if the queen is removed from the colony, female alates shed their wings and develop their ovaries (Fletcher and Blum, 1981a,b, 1983a; Vargo, 1999).

Along with the presence of the functional queen, additional factors may influence dealation of alates. For example, the age of alates and the presence of workers and brood can have an affect. Fletcher et al. (1983) reported that there were no significant differences between the numbers of overwintered and spring-reared alates that dealated in the presence of workers and brood; however, when isolated from workers and brood, a significantly larger number of overwintered alates dealated. Experimental procedures that can influence dealation include antennectomy (the surgical removal of the antennae) and topical applications of juvenile hormone analogues. Vargo and Laurel (1994) reported that female alates relieved of their antennae shed their wings in the presence of the functional queen, suggesting that the mode of perception of the inhibitory queen pheromone is through the antennae. In addition, non-antennectomized female alates that were topically treated with

methoprene dealated in the presence of the queen, suggesting that the inhibitory pheromone prevents wing casting of cohabiting female sexuals by suppressing the activity of the corpora allata (CA), the source of juvenile hormone (JH). It has been proposed that the pheromone does not halt JH secretion altogether, but reduces it to a level which allows a continuous but low rate of vitellogenin synthesis, thereby permitting some degree of ovary development; however, JH levels are not sufficient to induce dealation (Fletcher and Blum, 1983a).

Results from several studies (Kearney et al., 1977; Barker, 1978, 1979; Vargo and Laurel, 1994) indicated that JH analogue treatments administered to alates can have a positive effect on dealation, and increasing concentrations may hasten wing casting (Kearney et al., 1977; Barker, 1978, 1979). These results suggest that JH is the natural stimulant to dealation in *S. invicta*. However, to date, JH concentrations have not been measured in fire ant female sexuals to determine whether the hormone increases as these adults mature, thereby potentially affecting the rates at which sexually immature and mature alates shed their wings. Nonetheless, JH titers have been measured in some insects at different ages, and the behavioral and physiological changes resulting from topically treating these insects with JH compounds

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have been observed (Kramer, 1978; Huibregtse-Minderhoud et al., 1980; Huang et al., 1994). For example, results from studies with the European honey bee, *Apis mellifera,* indicate that older adult workers in queenright colonies produce higher amounts of JH compared with younger individuals (Rutz et al., 1976; Fluri et al, 1982), and topical or oral administration of either methoprene or hydroprene prematurely induces behaviors such as foraging (Robinson, 1985; Robinson and Ratnieks, 1987) and flight (Robinson and Ratnieks, 1987).

It may be possible that even under pheromonal control, adult female fire ant alates of different ages secrete different levels of JH, and once these alates are released from functional queen influences, the rates at which alates shed their wings is in accordance with their ages. This study will investigate the rates of dealation of sexually immature and mature female sexuals with and without workers and brood. In addition, the CA of sexuals of different ages will be measured to ascertain any variations in gland sizes as the ants mature, with a view to providing indirect data regarding the level of JH production.

Materials and Methods

Source of Alates and Colony Units. *Solenopsis invicta* monogyne colonies producing sexual brood were collected from north central Florida during the spring of 1997 and were separated from the soil by flooding (Jouvenaz et al., 1977). Each colony was divided into queenright and queenless units, each containing 6g of worker adults and 1g of worker brood. Female sexual pupae were distributed equally to units. Additional field-collected monogyne female sexual pupae were introduced into units deficient of sexual brood. Male adults and pupae were not used. Colony units were maintained in the laboratory at 27°C and 47% humidity. The colonies were fed a copious diet of crickets, sugar water, and tap water. Units were monitored every 12h in order to identify newly-eclosed female alates (11.8±2.1mg, n=70). The females were then marked with ballpoint industrial pens (Mark-Tex Corp., Englewood, NJ) in order to facilitate age identification of individual alates.

In queenright units, the antennae of newly-eclosed alates (n=17) were removed at the scape, close to the insertion point, with iridectomy scissors. In separate queenright units, alate controls (n=12) were amputated at a middle leg, near the proximal region of the femur (Vargo and Laurel, 1994).

In queenless units, newly-eclosed alates were antennectomized (n=19), while in the remaining queenless units, alates were leg-amputated (n=22) as described above. Alates were observed every 12h for indications of dealation, defined as the removal of at least three of their four wings. Female sexuals produce detectable amounts of the dealation inhibitory pheromone within three days of shedding their wings (Vargo, 1999); therefore, in this study, sexuals were removed immediately following wing casting.

Sexual maturity is reached within seven to ten days in female alates (Glancey, unpublished data, cited in Lofgren et al., 1975). The above procedures were repeated for 7-day-old (15.3±1.3mg, n=70) and 14-day-old alates (16.2±0.93mg, n=76).

Along with examining the rates of dealation among alates with workers and brood, wing casting was observed for alates that were in isolation. For this experiment, sexually mature monogyne alates were isolated individually (n=15) and in groups of threes (n=30) in test tubes (70ml) half-filled with moistened cotton. All tubes were sealed with cotton to prevent escape. Alates were observed every 12h for dealation.

In order to linearize the relationship between percent dealation and time of dealation, percents were converted to Probits. Data were then analyzed by Pearson chi-square goodness-of-fit tests.

Measurement of Corpus Allatum. The corpora allata of newly-eclosed and 14-day-old female alates and uninseminated sexually mature 19-day-old dealates were measured. The female was pinned dorsally through the thorax with a minutien pin in a wax-covered petri dish. The head was positioned by placing additional pins through each of the compound eyes. Dissections were conducted on the posterior portion of the ant's head. A scalpel was designed by fashioning the tip of a razor blade onto a wooden stick (12.5cm long, 3mm diameter), and the cuticle of the head was cut in a circular direction, clockwise from the right eye to the base of the head and counter-clockwise from the left eye to the base of the head. The cuticle was carefully peeled off with fine forceps. Anterior muscles and glands were excised, and the brain was lifted to gain access to the CA.

Once the CA were located, the brain was removed from the cuticle and placed on a microscope slide (Fig. 2-1). The area of an individual corpus allatum (Fig. 2-2) was determined by measuring the diameter of the gland with a stage micrometer. Corpora allata were measured in newly-eclosed (n=5) and 14-day-old female alates

(n=5) and 19-day-old uninseminated dealates (n=5). Preparation of one corpus allatum often resulted in the destruction of the second.

Results

Dealation of Sexually Immature and Mature Alates. The rates of dealation were observed for both antennectomized and non-antennectomized alates in the presence and absence of the functional queen. Over a 108-h observational period, non-antennectomized alates did not shed their wings in the presence of the functional queen. However, within each age category, 100% of alates that were liberated from queen pheromonal influences—either by removing the functional queen, antennectomizing the alates, or both—dealated by 108h.

Comparison of Probit slopes showed that within each age category, there were no significant differences ($P>0.05$) in the rates of dealation among antennectomized alates in the presence of the queen, antennectomized alates in the absence of the queen, and non-antennectomized alates in the absence of the queen. Though not significantly different, the rates of dealation of all

antennectomized alates were marginally faster than the rates of non-antennectomized alates in the absence of the queen (Figs. 2-3 to 2-5).

Comparison of dealation rates of sexually immature and sexually mature females showed that there were no significant differences ($P>0.05$) in dealation rates of antennectomized newly-eclosed, 7-day-old, and 14-day-old alates in the presence of the queen; additionally, there were no significant differences ($P>0.05$) among either antennectomized or non-antennectomized alates in the absence of the queen (Figs. 2-3 to 2-5).

Dealation of Isolated Alates. Within 156h, 100% dealation occurred with alates isolated individually and in groups. No significant differences existed ($P>0.05$) in dealation rates of the two sets of alates. However, the rates of dealation of alates in the presence of workers and brood were significantly faster ($P<0.05$) than those of alates separated from workers and brood (Fig. 2-6).

Measurement of Corpus Allatum. A one-way ANOVA test failed to show significant differences ($F_{2,13}=0.385$, $P>0.05$) in area measurements of the CA of sexually immature female alates ($40.12 \pm 0.98 um^2$), sexually mature female alates ($40.15 \pm 1.0 um^2$), and uninseminated sexually mature female dealates ($40.24 \pm 0.59 um^2$).

Discussion

Among social insects, primer pheromones produced by the colony's queen(s) play major roles in controlling the behavior and physiology of colony members (Wilson, 1971). In *Solenopsis invicta*, primary egg-laying queens produce a pheromone that inhibits dealation and ovary development in nestmate female alates (Fletcher and Blum, 1981a,b; 1983a). Fletcher and Blum (1981a,b; 1983a) proposed that the inhibitory pheromone suppresses JH production to a degree that permits vitellogenesis, whereas higher levels are necessary to induce dealation. The precise mode of action of JH suppression is not fully known.

With the use of topical treatments of JH I, II, and III, Kearney et al. (1977) demonstrated that JH induced dealation and that the rate of dealation of sexually mature female alates is dependent upon JH quantities. It is unknown whether fire ant alates produce different amounts of JH over their life time and whether these concentrations influence the rates at which pheromonally disinhibited alates shed their wings.

This study examined whether age/sexual maturity is a factor that influences the time at which female alates shed their wings when released from queen pheromonal influences, either by being separated from the primary queen or by being relieved of their

antennae. Dealation was examined for alates in the presence of workers and brood. The results of this study demonstrated that there are no significant differences in the rates of dealation of sexually immature and mature female sexuals that are released from queen pheromonal control, either by antennectomizing sexuals, removing the queen, or both (Figs. 2-3 to 2-5). If the functional queen reduces JH synthesis of nestmate alates to the point of preventing dealation, as has been suggested (Fletcher and Blum, 1983a; Vargo and laurel, 1994), and if the rate of dealation can be accelerated by topical JH treatments (Kearney et al., 1977), the findings from this investigation suggest that JH quantities in *S. invicta* female alates do not increase substantially with age. Therefore, once freed from queen pheromonal influences, sexually mature female alates do not shed their wings more readily than sexually immature alates. If JH concentrations must reach threshold levels in order for dealation to occur (Fletcher and Blum, 1983a; Vargo and Laurel, 1994), and if about 80% of alates shed their wings within about four days of disinhibition (Figs. 2-3 to 2-5), then the results of this study would suggest that the CA of female alates become active within four days of pheromonal liberation, regardless of sexual maturity.

This study supports the suggestion of Vargo and Laurel (1994) that the queen inhibitory pheromone is detected by sensory cells in the antennae; hence, when the antennae are removed, alates are not under pheromonal control, and dealation commences. The results of this investigation also revealed that, regardless of age, antennectomized alates in queenright and queenless colony units were induced to shed their wings sooner than non-antennectomized alates in queenless units, though the differences were not statistically significant (Figs. 2-3 to 2-5). Vargo and Laurel (1994) reported that in queenright colonies, antennectomized alates were stimulated to dealate significantly earlier than non-antennectomized alates; however, the rates of dealation of antennectomized and control alates were not significantly different in queenless colonies.

Along with examining the effects of age on dealation, this study investigated whether the exclusion of workers and brood influences the rate of dealation. Under natural conditions, female alates leave the colony and engage in a mating flight. After landing from the flight, inseminated female alates may be found in groups. Results from this study indicated that no differences existed between dealation rates of alates isolated individually and in groups; however, these rates were slower than those of alates in the

presence of workers and brood (Fig. 2-6). Fletcher and his colleagues (1983) also observed that disinhibited alates shed their wings more readily when workers and brood were present than alates not in the presence of workers and brood. Workers do not appear to physically assist in the dealation process, but results from this study, along with those of Fletcher et al. (1983), indicate that workers provide unknown stimuli for dealation. Tactile and olfactory stimuli and food are possible factors provided by workers and brood that may influence dealation.

In addition to comparing dealation rates of sexually immature and mature *S. invicta* female sexuals under different physical and social conditions, this study includes CA measurements of sexually immature and mature female alates and CA measurements of sexually mature uninseminated female dealates. Area measurements indicate that the sizes of the CA do not increase substantially with sexual maturity or with the absence of wings.

Though there is little evidence in the literature to correlate JH production with the sizes of the CA, changes in JH titers have been examined and compared with CA sizes in some insects. While no correlation was observed between CA sizes and their activity in *Schistocerca gregaria* (Tobe and Pratt, 1975, 1976), a relationship was reported in *Diploptera punctata* (Tobe and Stay, 1977).

The production of JH has been compared with the sizes of the CA in some insects that undergo complete metamorphosis. Röseler and his associates (1980) observed that in *Polistes dominulus*, the rate of JH biosynthesis correlates with the sizes of the CA. In *Leptinotarsa decemlineata*, Kramer (1978) examined the rates of JH synthesis in female adults under varying physiological conditions. He reported that changes in JH levels correspond to changes in the sizes of the CA (Schooneveld et al., 1977). However, Kramer (1978) noted that this correlation no longer exists after the first few days of adulthood.

In summary, this study indicates that there are no significant differences in the rates of dealation of pheromonally disinhibited sexually immature and mature *S. invicta* female alates in the presence of workers and brood; however, the rate of dealation is slower in alates that are removed from colony units, suggesting that workers and brood provide stimuli for dealation.

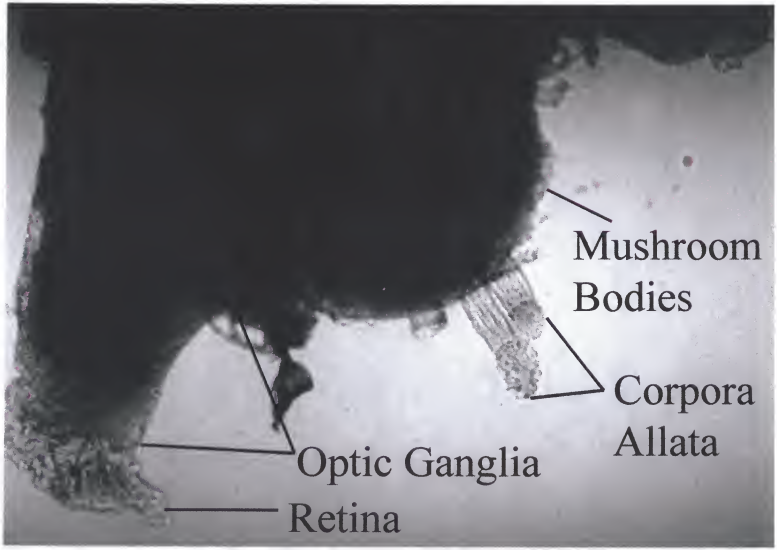

Figure 2-1. Brain of newly-eclosed *Solenopsis invicta* female alate.

Figure 2-2. Corpus allatum of newly-eclosed *Solenopsis invicta* female alate.

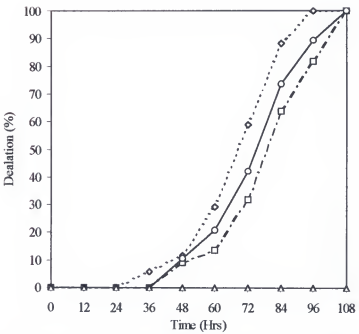

Figure 2-3. Rates of dealation of newly-eclosed female alates under four conditions: □-newly-eclosed alates without queen, n=22; ◇-antennectomized newly-eclosed alates with queen, n=17; ○-antennectomized newly-eclosed alates without queen, n=19; Δ-newly-eclosed alates with queen, n=12.

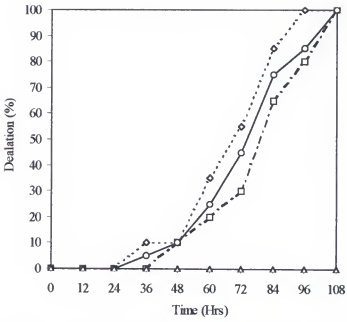

Figure 2-4. Rates of dealation of 7-day old female alates under four conditions: □-7-day old alates without queen, n=22; ◇-antennectomized 7-day old alates with queen, n=17; ○-antennectomized 7-day old alates without queen, n=19; Δ-7-day old alates with queen, n=12.

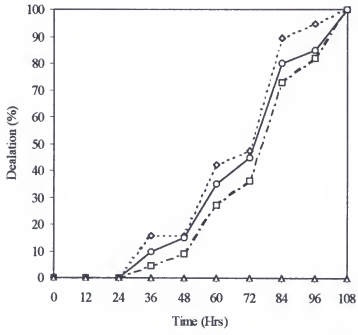

Figure 2-5. Rates of dealation of 14-day old female alates under four conditions: □-14-day old alates without queen, n=22; ◇-antennectomized 14-day old alates with queen, n=19; ○-antennectomized 14-day old alates without queen, n=20; Δ-14-day old alates with queen, n=15.

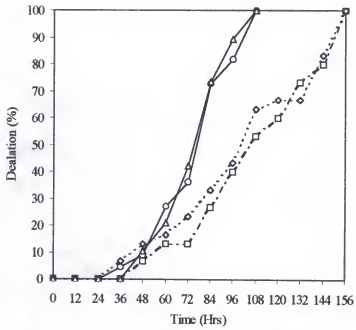

Figure 2-6. Rates of dealation of isolated and grouped alates: □-grouped alates, n=30; ◇-isolated alate, n=15; ○-grouped alates with workers and brood, n=30; Δ-single alate with workers and brood, n=15.

CHAPTER III

EFFECTS OF JUVENILE HORMONE III AND PRECOCENE II APPLICATIONS ON DEALATION OF *Solenopsis invicta* FEMALE ALATES

Introduction

While in the nest, *Solenopsis invicta* female alates are prevented from shedding their wings (dealating) and developing their ovaries by a queen inhibitory primer pheromone (Fletcher and Blum, 1981a,b, 1983a). However, topical applications of juvenile hormone (JH) I, II, and III (Kearney et al., 1977) or methoprene, a JH analogue, (Vargo and Laurel, 1994) administered to alates were shown to induce dealation and ovary development. This suggests that the queen inhibitory pheromone suppresses reproductive development in cohabiting female sexuals by regulating JH titers.

In 1976, Bowers and his colleagues discovered that chromenes extracted from the floss flower, *Ageratum houstonianum*, induced precocious metamorphosis in nymphs of the milkweed bug, *Oncopeltus fasciatus*. The chromenes also sterilized adult female milkweed bugs. The two compounds were named precocene I (7-methoxy-2,2-dimethyl chromene) and precocene II (6,7-dimethoxy-2,2-dimethyl chromene).

Precocenes have been shown to exert anti-JH effects in some holometabolous insects. For example, Kiguchi (1981) reported that precocene II administered to larvae of the commercial silkworm, *Bombyx mori*, induced precocious pupation, eliminating the final larval stage. Goewie et al. (1978, cited in Bowers, 1983) reported that treatments of precocene II on 90-hour-old queen larvae of the European honey bee, *Apis mellifera*, caused the development of worker-like intermediates. Goewie and his colleagues also found that applications of precocene resulted in allatal atrophy in the honey bee. However, Rembold et al. (1979) were unable to show an anti-JH effect in the honey bee.

The role of JH on dealation in *S. invicta* sexuals has been studied with the use of topical applications of JH (Kearney et al., 1977; Barker, 1978) and methoprene (Vargo and Laurel, 1994). Although not investigated, the potential effects of precocene on wing casting may provide additional evidence of the role of JH in regulating dealation in sexuals of the fire ant. This study investigates the role of JH on dealation in *S. invicta,* using topical applications of synthetic JH III and precocene II, and examines whether these treatments affect the sizes of the corpora allata (CA).

Materials and Methods

Source and Maintenance of Fire Ants. *Solenopsis invicta* monogyne colonies producing sexual brood were collected from north central Florida during the spring of 1997 and were separated from the soil by flooding (Jouvenaz et al., 1977). Each colony was divided into queenright and queenless units, each containing 10g of worker adults and 1.5g of worker brood. Ten sexually mature female alates (14.8±1.22mg) were distributed into each of the queenright and queenless units. Colony units were maintained in the laboratory at 27°C and 47% humidity. Ants were fed a copious diet of crickets, sugar water, and tap water.

Timing of Dealation with Juvenile Hormone Treatments. Each alate in a queenright unit was topically treated on the head with either 0.01ng JH III/ul acetone (n=30), 0.03ng JH III/ul acetone (n=30), 0.1ng JH III/ul acetone (n=30), 0.3ng JH III/ul acetone (n=30), 1ng JH III/ul acetone (n=30), or 1ul acetone (control, n=30). Each application administered was 1ul. Queenright units were observed every 12h to monitor dealation, defined as the removal of at least three of the four wings. Sexuals were removed immediately upon wing casting.

A separate group of alates in queenright units was topically treated on the head with either 20ng JH III/ul acetone (n=20) or 1ul

acetone (control, n=10). Each application administered was 1ul. These alates were observed every hour to monitor dealation.

In order to linearize the relationship between percent dealation and time of dealation, percents were converted to Probits. Data were then analyzed by Pearson chi-square goodness-of-fit tests.

Timing of Dealation with Precocene Treatments. Subsequent experiments examined the effects of precocene II on sexually mature female alates. Similar to the above procedures, each alate in a queenless unit was topically treated on the head with either 60ug precocene/ul acetone (n=30), 70ug precocene/ul acetone (n=30), 80ug precocene/ul acetone (n=30), 90ug precocene/ul acetone (n=30), 100ug precocene/ul acetone (n=30), or 1ul acetone (control, n=30). Each application administered was 1ul. Queenless units were examined in the same manner described above for queenright units.

Measurement of Corpus Allatum. The corpora allata of sexuals treated with 1ng JH/ul acetone (n=5), 100ug precocene/ul acetone (n=5), and 1ul acetone (n=5) were measured. The head was positioned dorsally in a wax-covered petri dish, and minutien pins were placed though the compound eyes. Dissections were executed on the posterior portion of the head of the ant. A scalpel

was designed by fashioning the tip of a razor blade onto a wooden stick (12.5cm long, 3mm diameter), and the cuticle of the head was cut in a circular direction, clockwise from the right eye to the base of the head and counter-clockwise from the left eye to the base of the head. The cuticle was then removed with fine forceps. Anterior muscles and glands were excised to better observe the brain. The brain was then lifted to acquire access to the CA.

Once the CA were located, the brain was excised from the cuticle and placed on a microscope slide. The area of an individual corpus allatum was determined by measuring the diameter of the gland with a stage micrometer.

Timing of Dealation With JH Rescue Treatments. Following observational experiments with precocene II applications, remaining precocene-treated alates in each of the queenless units were counted. Each unit that contained at least fifteen alates was divided into three sub-units with approximately equal amounts of alates, workers, and brood. An alate from a sub-unit was treated with either 10ng JH III/ul acetone (n=18), 20ng JH III/ul acetone (n=18), or 1ul acetone (control, n=18). Each application administered was 1ul. Units were observed every 12h to monitor dealation.

Results

Timing of Dealation With Juvenile Hormone Treatments. All JH-treated alates that were monitored at 12-h increments dealated within 108h of applying hormone (Fig. 3-3). Comparison of Probit slopes showed that alates treated with 1ng JH III shed their wings at a significantly higher rate ($P<0.05$) than alates given lower doses of JH III. One hundred percent dealation occurred within 24h in alates treated with 1ng JH III. Alates treated with 1ul acetone did not shed their wings during the 108-h observational period. There were no significant differences ($P>0.05$) in rates of dealation among alates given JH III treatments between 0.01 and 0.3ng (Fig. 3-3).

One hundred percent dealation occurred within 10h in alates treated with 20ng JH III. Observations taken every hour revealed that 55% dealation occurred within 5h. Alates administered 1ul acetone did not shed their wings during the 10-h observational period (Fig. 3-4)

Timing of Dealation With Precocene Treatments. Approximately 59% of precocene-treated alates residing in queenless colony units dealated within 108h (Fig. 3-5). Alates treated with 90 or 100ug precocene II retained their wings at a significantly higher rate ($P<0.05$) than those given lower doses of

precocene II. There were no significant differences ($P>0.05$) among dealation rates of alates given 60-80ug precocene II and 1ul acetone. However, 17 and 3% of alates treated with 90 and 100ug precocene II dealated within 108h, respectively. One hundred percent of alate controls shed their wings within the 108-h observational period (Fig. 3-5).

Measurement of Corpus Allatum. A one-way ANOVA test revealed that there was no significant difference ($F_{1,8}=0.611$, $P>0.05$) in area measurements of the corpora allata of dealates treated with 1ng JH III ($40.1\pm2.3um^2$, n=5) and alates treated with 1ul acetone ($39.98\pm1.0um^2$, n=5). However, there was a significant difference ($F_{2,13}=0.134$, $P<0.05$) between these CA measurements and those of alates treated with 100ug precocene II ($35.4\pm1.8um^2$, n=5).

Timing of Dealation With JH Rescue Treatments. Within 24h of applying 10 and 20ng JH III, 72 and 77% of alates shed their wings, respectively. Within 48h, 94% of alates treated with 10ng JH III shed their wings, while 100% of alates treated with 20ng JH III dealated. Alates treated with 1ul acetone did not shed their wings during the 108-h observational period (Fig. 3-6).

Discussion

The results of this study demonstrate that topical applications of JH III induce dealation in sexually mature *Solenopsis invicta* female alates while in the presence of the queen inhibitory pheromone. One hundred percent of alates topically treated with 20ng JH III were induced to shed their wings within 10h (Fig. 3-4), while applications of 1ng JH III stimulated 100% of alates to dealate within 24h (Fig. 3-3). Longer time periods were observed in alates treated with smaller doses. Similarly, Kearney et al. (1977) reported that within 24h, 98% dealation occurred among groups of female sexuals topically treated with 1ng JH III, whereas lower levels of dealation were observed with smaller doses. The present study shows that JH III applications ranging from 0.01 to 20ng were sufficient to stimulate alates to shed their wings while under the influence of the queen primer pheromone (Fig. 3-3). Vargo and Laurel (1993) reported that the JH-analoque methoprene (0.5ug/0.5ul acetone) also induced dealation while in the presence of the functional queen.

In an attempt to determine the importance of JH in dealation in the red imported fire ant, Barker (1978) found that allatectomized alates that were placed in queenless colonies failed to shed their wings after 28 days. Because of the difficulty of extracting the

corpora allata, especially during immature stages or during embryogenesis, precocenes, compounds extracted from the plant *Ageratum houstonianum*, have been used as chemical means of inactivating the CA in some insects, thereby disrupting normal endocrine functions (Bowers et al., 1976; Bowers, 1983; Müller et al., 1978).

Bowers et al. (1976) discovered that precocene I and II induced precocious metamorphosis in the milkweed bug, *Oncopeltus fasciatus*. In initial studies with the compounds, second instar milkweed bugs were exposed to the residue of the oily extract of *A. houstonianum*. The nymphs molted to apparently normal third and fourth instars but prematurely molted to tiny adults, thereby excluding the fifth instar.

Some holometabolous insects administered doses of precocene topically or orally exhibit abnormal development. For example, a delay in development was reported in the tobacco budworm, *Heliothis virescens*, the wax moth, *Galleria mellonella* (Bowers, 1983), and the yellow mealworm, *Tenebrio molitor* (Truman et al., 1973). In the commercial silkworm, *Bombyx mori*, precocene has been shown to cause precocious metamorphosis. In the silkworm, precocene induced early pupation, eliminating the last instar (Kiguchi, 1981).

Investigations of the effects of precocene in social insects have been very limited, and among the studies undertaken to determine whether precocene causes a response in the European honey bee, *Apis mellifera*, no consensus has been established. The results of the present study appear to reveal an anti-JH effect in *S. invicta* alates treated with precocene II. Applications of 90 and 100ug precocene II inhibited over 80% of alates from shedding their wings within 108h in queenless colonies. This was a significant reduction in dealation compared with 100% dealation within 10h in alates treated with 20ng JH (Figs. 3-3 to 3-6).

While no differences in the sizes of the CA were found in alates treated with 1ng JH III and those of alates treated with 1ul acetone (control), the CA of alates treated with precocene were substantially smaller than those of alates treated with JH III and acetone. The association of JH levels and the sizes of the CA have been investigated in some insects. For example, in *Schistocerca gregaria*, the CA of locusts producing low levels of JH are not significantly smaller than those of locusts producing larger amounts of JH (Tobe and Pratt, 1975, 1976). However, in the beetle *Leptinotarsa decemlineata* (Schooneveld et al., 1977) and in the cockroach *Diploptera punctata* (Holbrook et al., 1997), the CA

sizes correspond with JH activity. In addition, in the female wasp *Polistes dominulus*, the rate of JH production is related to the sizes of the CA (Röseler et al., 1981). In the present study, JH titers in *S. invicta* alates were not measured; therefore, a comparison of CA sizes with JH production could not be determined. However, the CA of precocene-treated alates were significantly smaller than those of alate controls. Within the CA, precocene produces 3,4-epoxy derivatives that react with surrounding proteins, resulting in necrotic atrophy of the CA (Wawrzenczyk, 1997).

Bowers et al. (1976) demonstrated that in *O. fasciatus*, the antiallatotropic activity of precocenes can be reversed with treatments of JH. The precocious maturation of nymphs treated with precocene II was halted with applications of JH III. In addition, Barker (1978) found that *S. invicta* alates surgically removed of their CA did not dealate in queenless colonies, but topically treating these alates with a synthetic mixture of 10ug JH stimulated 100% of allatectomized alates to shed their wings within 36h. In the present study, alates chemically allatectomized with precocene II (Bowers et al., 1976; Tobe and Stay, 1986) were stimulated to shed their wings with JH III. As much as 94 and 100% dealation occurred within 48h of applying 10 and 20ng JH III, respectively (Fig. 3-6).

The reduction in CA sizes of precocene-treated sexuals, along with the effects of JH and precocene on dealation, suggests that JH is the natural stimulant to wing casting in the red imported fire ant. In addition, topical applications of JH used in this study, ranging from 0.01-20ng, were sufficient to overcome the inhibitory effects of the queen primer pheromone, indicating that the pheromone prevents wing casting by suppressing JH levels. Fletcher and Blum (1983a) proposed that the queen inhibitory pheromone does not cease the secretion of JH, but lowers it to a point that causes a continuous but low rate of vitellogenin synthesis, hence allowing sexual maturation; however, JH levels are not sufficient to cause alates to shed their wings.

The effects of topical applications of JH III and precocene II on dealation in *S. invicta* alates, along with the effects of these compounds on the sizes of the CA of female sexuals, provide supporting evidence of the role of JH in dealation.

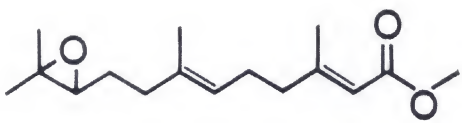

Figure 3-1. Molecular structure of juvenile hormone III.

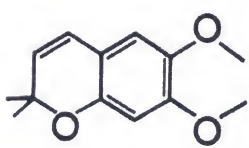

Figure 3-2. Molecular structure of precocene II.

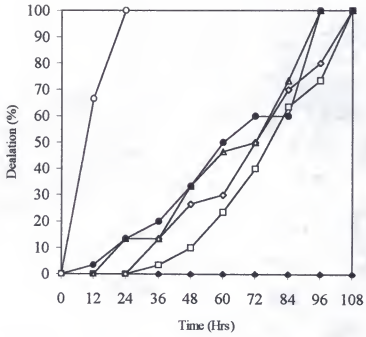

Figure 3-3. Rates of dealation of sexually mature *S. invicta* female alates in queenright colonies following topical applications of JH III and acetone: ♦-1ul acetone, n=30; □-0.01ng JH/ul acetone, n=30; ◇-0.03ng JH/ul acetone, n=30; Δ-0.1ng JH/ul acetone, n=30; ●-0.3ng JH/ul acetone, n=30; ○-1ng JH/ul acetone, n=30.

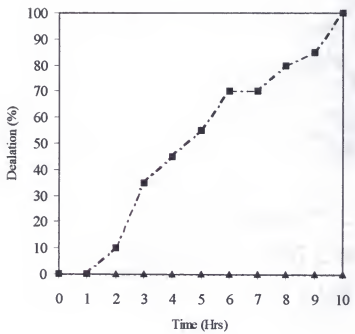

Figure 3-4. Rates of dealation of sexually mature *S. invicta* female alates in queenright colonies following topical applications of ▲-1ul acetone, n=10, and ■-20ng JH III/ul acetone, n=20.

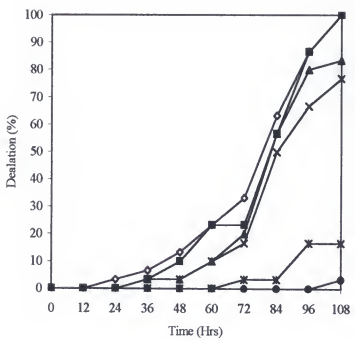

Figure 3-5. Rates of dealation of sexually mature *S. invicta* female alates in queenless colonies following topical applications of precocene II and acetone: ◇-1ul acetone, n=30; ■-60ug precocene/ul acetone, n=30; ▲-70ug precocene/ul acetone, n=30; ×-80ug precocene/ul acetone, n=30; ∗-90ug precocene/ul acetone, n=30; ●-100ug precocene/ul acetone, n=30.

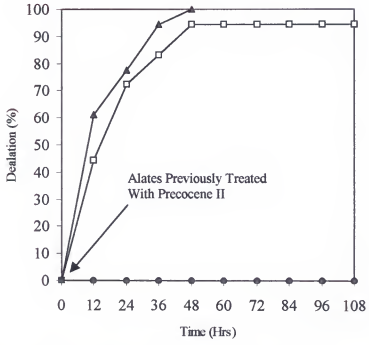

Figure 3-6. Rates of dealation of precocene-treated *S. invicta* female alates in queenless colonies following topical applications of acetone and rescue treatments of JH III: ●-1ul acetone, n=18; □-10ng JH/ul acetone, n=18; ▲-20ng JH/ul acetone, n=18.

CHAPTER IV

THE ROLE OF JUVENILE HORMONE IN INDUCING *Solenopsis invicta* FEMALE ALATES TO FLY

Introduction

Insecta is the most abundant class of animals on earth, both in terms of number and diversity (Evans, 1984). Insects have exploited numerous environmental habitats, ranging from deserts to snowy domains and from freshwater rivers to hot springs. The success of insects can be attributed to their morphological, behavioral, and physiological means of adapting to diverse conditions (Goldsworthy and Wheeler, 1989). Some advantageous characteristics that insects possess include a small size, a hard exoskeleton, and a high reproductive potential (Evans, 1984). Along with these and other attributes, the ability to fly has contributed greatly to their success. Flight has enabled insects to disperse rapidly, obtain food, escape from enemies, and search for mates (Goldsworthy and Wheeler, 1989; Starr and Taggart, 1989).

Flight is essential to mating in *Solenopsis invicta*. Female and male reproductives mature in the colony until suitable weather conditions stimulate them to engage in a mating flight (Vinson and Sorenson, 1986). These flights normally commence in early

afternoon, one or two days following rain (Milio et al., 1988). Markin et al. (1971) reported that flights usually occur when temperatures are between 20° and 32°C, and wind gusts generally do not exceed 5mph. Rhoades and Davis (1967) found that the minimum humidity during the flight is 80%.

Before the mating flight takes place, workers form exit holes on the surface of the mound and display an alarm-like behavior (Markin et al., 1971; Obin and Vander Meer, 1994) that is induced by mandibular gland secretions from the female and male reproductives (Alonso and Vander Meer, 1997). Usually males commence flying before females. Males arise from the nest, climb onto nearby vegetation, and eventually fly into the air. Afterwards, female alates emerge and ascend into the swarm of males in the air (Milio et al., 1988). A thousand or more alates have been reported leaving a single mound for a mating flight (Markin et al., 1971; Rhoades and Davis, 1975). A female alate will acquire a lifetime quantity of sperm from a male during the flight (Ross and Fletcher, 1985). Males die after mating, while inseminated females continue flying in search of reflective or moist surroundings on which to land and establish a colony (Markin et al., 1973; Vinson and Sorensen, 1986).

In some insects, juvenile hormone (JH) plays a major role in flight behavior. A number of studies have been undertaken to determine the effects of JH and precocene on flight in insects (see Rankin, 1989 and 1991 for reviews). For example, Robinson and Ratnieks (1985, 1987) reported that topical and oral treatments of the JH analogue methoprene in the European honey bee, *Apis mellifera*, caused premature flight activity. Similarly, Rankin and Rankin (1980) demonstrated that the JH mimic altosid, topically applied to female and male ladybird beetles, *Hippodamia convergens,* significantly increased flight duration. Precocene II treatments reduced the duration of flight in both sexes of the beetles, and re-administering altosid reversed this inhibition.

With the use of topical precocene and JH applications, this study investigates the role of JH in inducing flight in *S. invicta* female alates.

Materials and Methods

Colony Maintenance and Topical Treatments on Alates. *Solenopsis invicta* monogyne colonies possessing numerous female sexual adults were collected from north central Florida during the spring of 2000. Ants were separated from soil by spreading a thin layer (less than 1cm deep) of colony soil into fluon-lined trays

(47.5cm long, 34.5cm wide, 11.5cm deep) and placing four petri dishes (10cm diameter, 2cm deep), layered with castone, in each tray. The bottom of each petri dish was moistened with water, entrance holes for the ants were drilled around the side of the dish, and red cellophane was used to cover the lid of the dish. Crickets and water were supplied to ants. Water was delivered in test tubes (two 14-ml tubes/tray) plugged with cotton. Colonies were left undisturbed overnight in the laboratory at 27°C and 47% humidity.

Once ants were separated from the soil, sexual brood were removed from each colony. Following a 9-day period, remaining ants were placed in fluon-lined buckets (25cm diameter, 25cm deep) filled with moist soil. Ant distribution for each bucket consisted of a functional queen, 5g of worker adults, and 0.5g of worker brood of the same colony.

Three groups of 40 sexually mature female alates (14.4±1.21mg) were each placed in a bucket of ants from their original colony. Alates from one group were each topically treated on the head with 100ug precocene II/ul acetone, and alates from a separate group (control 1) were each topically treated with 1ul acetone and placed in a different bucket. Each application administered was 1ul. The remaining alates (control 2) were

untreated and placed with ants from their colony. All ants were undisturbed for four days to allow acclimation to soil and for treatments to adsorb through the cuticle of alates (see Chapter III).

Induction of Mating Flight. Following the four-day acclimation period, alates were induced to fly (Markin et al., 1971; Milio et al., 1988; Obin and Vander Meer, 1994). First, three tongue depressors were pushed approximately halfway into the dirt at an angle in each bucket in order to simulate vegetation onto which alates climb prior to flying. A flexible incandescent lamp with a 40W bulb served as a light source and was placed directly over the collection of colonies, and mists of water were sprayed onto soil to imitate rain. Female alates observed crawling onto the tongue depressors were collected and placed in a fluon-lined cup (265.5ml).

Tethering of Female Alates. Each precocene-treated (n=15), acetone-treated (n=15), and untreated (n=15) alate was prepared for tethered flight by gluing (Future Glue, Pacer Technology) a tiny loop (approximately 0.05cm diameter) of fine silver wire (0.002cm diameter) onto the prothorax and extending the wire at an angle about 5cm from the loop. Next, the alate was warmed for ten seconds by placing the ant 5-10cm from the 40W bulb. Afterwards, flight induction consisted of waving the alate into the air several

times with jeweler's forceps (11cm long) and blowing about five seconds of light puffs of air underneath the wings, toward the base (Fig. 4-1). Previous observations have shown that tethered alates are less likely to be induced to fly after being stimulated for more than 10min. Therefore, in this study, attempts to stimulate flight endured for only 10min for each alate. Once flight was induced, wire extending from the alate was mounted onto a piece of modeling clay attached to a ring stand (Fig. 4-2). The alate was positioned about 5cm from the light source. An alate that ceased to fly was stimulated by quickly blowing puffs of air underneath the wings. For this study, a successful flight was defined as having a duration of 5min, including time needed to stimulate alates that had temporarily ceased flying.

Flight With JH Rescue Treatments. None of the alates treated with 100ul precocene II/ul acetone were observed climbing tongue depressors, and these treated alates could not be stimulated to fly. In order to have sufficient alate subjects, two colonies were each placed in a bucket. As described above, each bucket contained a functional queen, 5g of worker adults, and 0.5g of worker brood. Forty untreated alates, originating from the two colonies (20 alates/colony), were each topically treated with 100ug precocene II/ul acetone on the head. These treated alates were

placed with ants from their respective colonies, and ants were left undisturbed for four days.

Following the four-day period, precocene-treated alates from each bucket were collected from the soil by spreading a thin layer of colony soil into fluon-lined trays. Alates from one bucket were each topically treated on the head with 20ng JH III/ul acetone, and each of the alates in the second bucket was treated with 1ul acetone (control). Each application administered was 1ul. Sixty-one percent dealation was observed within 12h of topically applying 20ng JH III onto precocene-treated alates (see Chapter III); therefore, in this study, JH-treated (n=10) and acetone-treated (n=10) alates were induced to fly 3-6h after applying chemicals.

Results

Flight Activity With Topical Treatments. *Solenopsis invicta* females were each treated with 100ug precocene II/ul acetone or 1ul acetone (control 1) and placed in buckets, each containing a functional queen, workers, and brood. Ants were induced to engage in mating flight behaviors (Markin et al., 1971; Milio et al., 1988; Obin and Vander Meer, 1994). Excited workers from the colony containing acetone-treated alates and those from the colony with untreated alates (control 2) were observed creating exit holes for

alates, and alates from the two colonies were seen crawling onto tongue depressors. However, workers in the colony with precocene-treated alates were not observed displaying mating flight behaviors, and alates were not seen crawling onto tongue depressors.

Eighty-seven percent of alates treated only with acetone and 93% of untreated alates could be induced to fly for 5min. There was no significant difference ($P>0.05$) in the proportion of acetone-treated alates stimulated to fly and that of untreated alates. However, none of the alates treated with precocene II could be stimulated to fly (Fig. 4-3). In addition, none of the precocene-treated alates could be induced to fly following JH or acetone applications.

Discussion

The results of the present study suggest that the reported cytotoxic effects of precocene II applications on the corpora allata (Bowers, 1985) of *Solenopsis invicta* female alates significantly lowered the percentage of tethered females taking flight compared with those of controls (Figure 4-3). Though not investigated, the absence of mating flight behaviors in workers (Markin et al., 1971; Milio et al., 1988; Obin and Vander Meer, 1994) contained in buckets with precocene-treated alates may originate from

insufficient release of mandibular gland chemicals that are normally produced from alates and used to induce worker excitement (Alonso and Vander Meer, 1997). The inhibitory effects of precocene on the secretion of JH (Bowers, 1985) may be indirectly responsible for suppressing worker behavior by reducing or eliminating natural chemical cues from alates.

Earlier studies (see Chapter III) revealed that 20ng JH III topically applied to precocene-treated alates in queenless colonies was sufficient to induce dealation (61%) within 12h, while acetone-treated controls did not shed their wings within 108h. However, in this study, 20ng JH III was not sufficient to reverse flight inhibition in alates treated with 100ug precocene II.

The failure of precocene-treated alates to display natural pre-flight behaviors (Markin et al., 1971; Milio et al., 1988; Obin and Vander Meer, 1994) and to take flight suggests that JH is necessary in stimulating flight, as well as pre-flight excitement. As noted, 20ng JH has been shown to have a positive effect on dealation in precocene-treated alates within 12h (see Chapter III), but the ineffectiveness of this JH dose in overcoming the inhibition to fly may be due to insufficient time (3-6h) allowed for the hormone to enter into the body. Increasing the time during which alates are exposed to the JH treatment before flight stimulation may demonstrate that

20ng JH is an adequate amount to reverse flight restraints. Or a combination of elevating the quantity of JH treatment and increasing the exposure period to the hormone before flight induction may provide positive results in inciting flight in precocene-treated females.

Fletcher and Blum (1983a) proposed that the functional queen suppresses JH titers, to some extent, in cohabiting female alates, thereby preventing dealation but allowing some degree of ovary development. It is unknown whether JH levels in female alates increase during the mating flight, thereby inducing dealation following insemination (Hölldobler and Wilson, 1990). A sensitive assay that measures any changes in JH titers in alates before and after a mating flight would provide substantial data regarding the endocrinology of *S. invicta*.

Figure 4-1. Flight stimulation of tethered *Solenopsis invicta* female alate.

Figure 4-2. Tethered alate in flight.

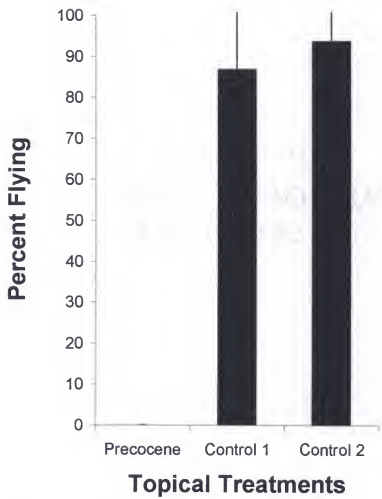

Figure 4-3. Pecentages of *S. invicta* female alates flying for five minutes under three conditions--topical treatment of 100ug precocene/ul acetone (precocene, n=15), topical treatment of 1ul acetone (control 1, n=15), and untreated (control 2, n=15)

CHAPTER V

AN INVESTIGATION OF MATING FLIGHTS TO DETERMINE WHETHER PRE-MATING ACTIVITIES CONTRIBUTE TO POST-MATING DEALATION

Introduction

Solenopsis invicta alates remain in the nest until proper conditions initiate a mating flight. Flights normally take place one or two days after a rain with temperatures ranging between 20° and 32°C, with a minimum humidity of 80%, and wind gusts that usually do not exceed 5mph (Rhoades and Davis, 1967; Markin et al., 1971; Milio et al., 1988).

Prior to the mating flight, workers form exit holes on the surface of the mound and exhibit an alarm-like behavior (Markin et al., 1971; Obin and Vander Meer, 1994) that is encouraged by mandibular gland secretions from the female and male reproductives (Alonso and Vander Meer, 1997). Males emerge from these holes before female alates, and the males climb onto nearby vegetation and commence flying (Rhoades and Davis, 1967). Milio et al. (1988) observed females leaving the nest within half an hour after males, and the duration of flight activity of these females was about 20min. With the aid of nets attached to an airplane, Markin and his

colleagues (1971) reported that most female alates were captured at heights of approximately 60-120m. Males were usually collected at 150m; however, some males were found to fly as high as 300m.

Males die once they have mated, but inseminated females descend to the ground, dealate, search for suitable nesting areas, and begin egg production (Toom et al., 1976; Noble, 1998). Fats (Vinson and Sorenson, 1986) and proteins (Jones et al., 1981) acquired from the breakdown of flight muscles are used for the survival of the females and for the production of eggs. A significant advantage of remaining in the nest and utilizing converted body tissues for nutrients (claustral colony founding) is that these newly-inseminated females decrease their risks of mortality from potential predators outside the nest (Schmid-Hempel, 1984).

Previous studies (see Chapter II) involving dealation with female alates that were removed from the queen, workers, and brood showed that 53% of alates maintained in groups and 63% of alates in isolation shed their wings in about 108h. However, in newly-mated females, dealation occurs within 4h after descending from the mating flight (Markin et al., 1972). The time in which these mated females are induced to shed their wings is significantly shorter than that of uninseminated females in queenless colonies. Topically treating female alates in queenright colonies with 20ng

juvenile hormone III (JH) induced 55% of alates to shed their wings within 5h, while longer time periods were detected with smaller doses of the hormone (see Chapter III). In addition, only 3% of alates administered 100ug precocene II, a compound shown to inhibit the secretion of JH in the corpora allata (CA) (Bowers, 1985), dealated within 108h in queenless colonies (see Chapter III). The results from these studies, using topical applications of JH III and precocene II, suggest that dealation can be induced by JH. However, it is unknown whether external factors like mating flight activities and/or environmental factors associated with the flight participate, in some manner, in elevating natural JH titers in newly-mated females, thereby stimulating dealation.

Specific insect behaviors have been reported to play important roles in regulating JH. For example, in the North American Monarch butterfly, *Danaus plexippus plexippus*, JH-sensitive reproductive organs are less developed in migrating females and males (Urquhart, 1960). Lessman and Herman (1981) reported that flight enhanced JH inactivation in the Monarch. They found that the hemolymph of Monarchs that had flown for 40min under laboratory conditions contained significantly higher levels of JH-specific esterases than butterflies that had not flown. Lessman and Herman (1981) proposed that reduced reproductive development in migrating

Monarchs may be the result of flight-induced JH breakdown, thereby preventing inappropriately-timed reproduction.

Huang and Robinson (1992) hypothesized that in the European honey bee, *Apis mellifera*, worker interaction influences JH titers and behavioral development. An earlier study (Robinson, 1987) reported that JH levels were lower in nurses than in older workers, foragers, and that JH administered to nurses induced early foraging. Huang and Robinson (1992) found that nurses isolated from foragers exhibited precociously high JH titers and engaged in foraging activities. It was proposed that under natural circumstances, foragers inhibit JH production in young workers, and as the older bees die, younger workers receive less inhibition, thereby promoting behaviors associated with older bees.

Inseminated *S. invicta* females returning from a mating flight are induced to shed their wings significantly earlier (Markin et al., 1972) than uninseminated females in queenless colonies (see Chapter III). JH has not been proven to be the physiological inducer to dealation in female reproductives; however, alates topically treated with JH III (see Chapter III) were stimulated to shed their wings in a time period comparable to that of newly-mated females. The potential elevation in JH titers in inseminated females may be responsible for post-mating dealation, and behaviors associated

with the mating flight may mediate, in some way, JH levels. This investigation examines whether pre-mating activities in *S. invicta* alates contribute to dealation.

Materials and Methods

Colony Maintenance. All *Solenopsis invicta* monogyne colonies used in this study originated from north central Florida. Alates under investigation were acquired directly from mounds and from collected colonies maintained in the laboratory.

Buckets (25cm diameter, 25cm deep) were used to contain laboratory-maintained colonies. To simulate vegetation onto which alates climb prior to the mating flight, three tongue depressors were pushed halfway into the dirt at an angle. Crickets and water were supplied to ants in each bucket. Water was delivered in test tubes (two 14-ml tubes/tray) plugged with cotton. Ants were left undisturbed overnight in the laboratory at 27°C and 47% humidity to allow acclimation to nest conditions.

Induction of Mating Flight. Colony ants were induced to engage in mating flight activities (Markin et al., 1971; Milo et al., 1988; Obin and Vander Meer, 1994). First, buckets filled with colony ants and soil were taken outside of the laboratory and placed directly in the sunlight (33±0.58°C and 52±2.3% humidity). Next,

mists of water were sprayed on colony soil for about 5min, and excited workers were observed creating exit holes about 30min after spraying. Alates emerged from the soil 30-60min following the onset of the formation of exit holes.

The rates of dealation were observed for alates (14.6±0.68mg, n_{total}=54) displaying a variety of behaviors associated with a mating flight. Five categories of alate behavior were examined: 1) excited alates scurrying on the surface of the soil (n=12, Fig. 5-1); 2) excited alates climbing onto tongue depressors (n=12, Fig. 5-2); 3) tethered alates flying for 5min under laboratory conditions but not primed for a mating flight (see Chapter IV for tethering and flight descriptions, n=9, Fig. 5-3); 4) alates displaying all of the identified pre-mating behaviors (n=9); and 5) alates not displaying any of the identified pre-mating behaviors (n=12, control). Alates displaying similar behavior(s) were assembled into groups (3 alates/group) and placed in a test tube (70ml) half-filled with moistened cotton. Alates were maintained outside of the laboratory for the duration of the experiment. Alates were observed every 12h for indications of dealation (at least three wings removed).

Dealation percents were converted to Probits to linearize the relationship between percent dealation and time of dealation.

Pearson chi-square goodness-of-fit tests were then applied to analyze data.

Results

All experimental alates shed their wings within 168h. Fifty-six percent of alates exhibiting all three pre-mating behaviors (scurrying, climbing, and flying) shed their wings within 108h. Over 50% of alates displaying only one (scurrying, 67%; flying, 56%) or two (scurrying and climbing, 75%) behaviors dealated by 120h. Nonetheless, a comparison of Probit slopes revealed that there were no significant differences ($P>0.05$) among the dealation rates of alates displaying various types of pre-mating behaviors. In addition, these rates did not differ statistically ($P>0.5$) from that of non-stimulated alates (Fig. 5-4). Results also indicated that during the 168h period, natural variations in temperature (ranging from 22±1.8°C to 35±1.4°C) and humidity (ranging from 45±3.6% to 94±2.3%) did not significantly ($P>0.05$) affect the rates of dealation compared with that of alates under laboratory conditions (27°C and 47% humidity, see Chapter II).

Discussion

Newly-mated *Solenopsis invicta* queens are induced to shed their wings within 4h (Markin et al., 1972), while uninseminated female alates in isolation or in groups may take up to 156h to dealate (see Chapter II). The stimulus to dealate early in mated queens may be the result of behavioral stimulation from activities associated with a mating flight. This study examined several pre-mating behaviors/activities associated with the flight to determine whether an individual pre-mating behavior (scurrying atop the soil or flying) or the combination of behaviors is critical to wing casting in female alates. Results indicated that pre-mating behaviors did not contribute significantly to dealation. Over 50% of alates exhibiting one or more behaviors shed their wings by about 120h, and some alates took up to 168h to shed their wings, a significant delay compared with newly-mated females.

Previous investigations have demonstrated that topical applications of JH III, ranging from 0.01-20ng, were sufficient to stimulate dealation in uninseminated *S. invicta* female reproductives in queenright colonies (see Chapter III). Hormonal induction of wing casting in newly-mated queens was not investigated in the present study; however, successfully identifying external factors (for example, behavioral and/or environmental) contributing to the

stimulation of dealation and ascertaining the physiological mode of action of these external factors on dealation may reveal the existence of an interplay with JH. For example, specific behaviors have been found to mediate JH regulation in some insect species. For example, Lessman and Herman (1981) reported that in *Danaus plexippus plexippus*, long-term flight (at least 40min) significantly increased the production of JH esterases, thereby breaking down JH compounds. And in *Apis mellifera*, Huang and Robinson (1992) hypothesized that the interaction among workers of different ages may affect JH production and division of labor. Foragers, the eldest workers in the colony, are believed to inhibit precocious development in young workers by suppressing JH levels. The mechanism by which the inhibition is relayed has not yet been determined but may be chemical or behavioral (Huang and Robinson, 1992).

Mating, alone or in combination with other behavioral and/or environmental cues, cannot be overlooked as a possible major factor in stimulating dealation in *S. invicta* females. In a number of insect species, mating is responsible for activating and enhancing egg development, and in some examples, these effects are credited to a rise in JH (see Davey, 1983 for review). Mating may be responsible for stimulating the CA through signals

transmitted by nerve connections. This can be best illustrated in some species of Dictyoptera. For example, mating activates the CA in *Diploptera punctata* (Engelmann, 1959; Stay and Tobe, 1977) and *Periplaneta americana* (Pipa, 1986) by removing inhibition from the nervi corporis allati. Mating may also involve the direct transfer of JH from the male to the female. *Hyalophora cecropia* males have been reported to transfer JH directly to females (Shirk et al., 1979). Along with the deposition of JH substances into the female, mating may have an allatotropic effect in females, thereby initiating JH biosynthesis (see Ramaswamy et al., 1997 for review). In *Heliothis virescens*, allatotropic stimuli in the female may accompany male transfer of JH in order to enhance egg production (Park et al., 1998).

Behavioral factors that may contribute to post-mating dealation in *S. invicta* alates could not be identified in this study. The difficulties of successfully force-copulating *S. invicta* reproductives (Toom et al., 1976; Ball et al., 1983; Cobey, 1998) prevented determining whether mating is a major stimulant, possibly by increasing JH levels in some manner. Environmental factors that were not taken into account, like height of flight and wind speed during flight, may also be important to dealation. In addition, tactile cues from the male during the flight have to be considered as

possible stimulants. Nonetheless, this investigation provides evidence as to the role of some of the most documented (Rhoades and Davis, 1967; Markin et al., 1971; Milio et al., 1988; Alonso and Vander Meer, 1997) and, to some degree, readily-induced pre-mating behaviors on dealation. Further investigation may eventually disclose important factors associated with the mating flight that encourage dealation and their role, if any, in increasing JH titers.

Figure 5-1. Excited alates and workers atop soil.

Figure 5-2. Alates preparing for flight.

Figure 5-3. Alate in flight.

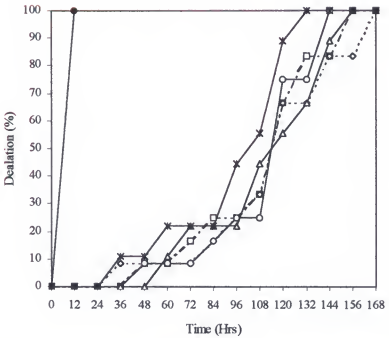

Figure 5-4. Rates of dealation of *S. invicta* female alates exhibiting different pre-mating behaviors: ◇-alates scurrying atop soil, n=12; ○-alates climbing tongue depressors, n=12; Δ-non-stimulated alates flying (lab-induced), n=9; ×-alates scurrying, climbing, and flying (lab-induced), n=9; □-non-stimulated alates (controls), n=12; ●-alates mating (Markin et al., 1972).

CHAPTER VI

IDENTIFICATION AND QUANTITATION OF JUVENILE HORMONE III IN *Solenopsis invicta* FEMALE ALATES

Introduction

Juvenile hormone (JH) is vitally important in the control of insect development and reproduction (Denlinger, 1985; Hoffmann and Lagueux, 1985; Pener, 1985; Raabe, 1989; Nijhout, 1994). Wigglesworth's (1934, 1936, 1940) classic experiments with *Rhodnius prolixus* were the first to demonstrate the existence of JH in the corpora allata (CA) and the importance of JH in development. Wigglesworth (1934, 1936) found that decapitated third-instars of *R. prolixus* did not molt into fourth-instars. Instead, the insects developed into adults. The implantation of active CA into the bodies of headless early-instars caused these immatures to molt to normal instars rather than to adults; however, implanting active CA into fifth-instars, which would normally molt to adults, resulted in the formation of giant sixth-instars. Wigglesworth (1940) reported that the factor inhibiting metamorphosis was secreted by the CA and was named "juvenile hormone." He found that the CA become active in early-instars and inactive in final-instars, thereby producing adults.

Wigglesworth (1936) also demonstrated that the CA of *R. prolixus* become active again in adults.

Röller et al. (1967, cited in Nijhout, 1994) were first to elucidate the chemical structure of JH as a sesquiterpenoid consisting of a methyl ester on one end and an epoxide group near the other. Juvenile hormone was later found to exist in seven structurally-related molecular homologs in insects. These JH compounds are identified as JH 0, JH I, JH II, JH III, JH III bisepoxide, 4-methyl JH I, and methyl farnesoate (Fig. 6-1). The most prevalent of the JH homologs is JH III, which has been identified in Coleoptera, Dictyoptera, Diptera, Hemiptera, Hymenoptera, Isoptera, Lepidoptera, and Orthoptera. Juvenile hormone 0, JH I, JH II, JH III, and 4-methyl JH I have been reported in Lepidoptera, with JH 0 and 4-methyl JH I found only in Lepidoptera eggs (see Gupta, 1990 and Nijhout, 1994 for reviews). Juvenile hormone III bisepoxide has been identified in Diptera (Rickards and Thomas, 1993; Yin et al., 1995). Other insect orders, including Dictyoptera (Lanzrein et al., 1984), Diptera (Borovsky et al., 1992), and Hemiptera (Feldaufer et al., 1982) have been shown to possess methyl farnesoate.

The isolation and quantitation of JH compounds have provided valuable information as to the roles of these compounds in

mediating physiological changes and behaviors. For example, JH III has been identified in the European honey bee, *Apis mellifera*, and may play a role in age polyethism. Juvenile hormone titers have been reported to exist in lower concentrations in young workers, nurses, than in older workers, foragers (Robinson, 1987). However, nurses that are isolated from foragers possess unnaturally high JH titers and initiate foraging activities, suggesting that the interaction of foragers and nurses prevents precocious behavioral development in nurses by lowering JH production (Huang and Robinson, 1992). In addition, the development of the hypopharyngeal glands in *A. mellifera* may be under the control of JH (Sasagawa et al., 1989). These exocrine glands are at their largest in nurses, and materials from the glands are fed to brood. The high degree of development of the hypopharyngeal glands has been shown to be associated with low JH biosynthesis, and the administration of synthetic JH to workers induces premature degeneration of the glands (Huang et al., 1994).

To date, JH compounds have not been identified in the red imported fire ant, an insect that possesses the same degree of social complexity as honey bees (Michener, 1974; Fletcher and Ross, 1985). Nonetheless, studies with *Solenopsis invicta* female alates that were allatectomized and/or administered JH suggest that

dealation (Kearney et al., 1977; Barker, 1978, 1979; Vargo and Laurel, 1994, see Chapter III), flight (see Chapter IV), and ovary development (Barker, 1978, 1979; Vargo and Laurel, 1994) are JH-controlled. It has been proposed that the functional queen suppresses JH titers in cohabiting alates, thereby suppressing wing casting and egg development, but this inhibition is released once alates are removed from the queen (Fletcher and Blum, 1981a; Vargo and Laurel, 1994).

The physicochemical methods of gas chromatography-mass spectroscopy (GC-MS) are extremely accurate in the identification and quantification of JHs (Raabe, 1989; Goodman et al., 1993; Teal et al., 2000). Bergot et al. (1981) developed the most commonly used technique for GC-MS in which the monoepoxide homologs of JH are converted to their corresponding methoxyhydrin derivatives and examined by electron impact MS with selective ion monitoring (SIM). The employment of GC-MS-SIM in the identification and quantitation of JH homologs entails the recognition of the relative retention index for each homolog derivative and the identification of the base peak for each homolog. This method is usually reliable; nonetheless, identifying and quantifying a homolog based on the examination of one fragment ion can be deceiving, especially when JH compounds have not been

detected in an insect species that is predicted to possess more than one homolog. Accuracy in detecting and quantifying JH homologs is enhanced by monitoring multiple ions with SIM. However, the availability of useful diagnostic ions is limited when using electron-impact MS because of low relative abundances and reduced sensitivity from monitoring large numbers of ions. Though the conversion of JH to methoxyhydrin derivatives is relatively simple, the reaction lowers recovery rates by 15-25% (Bergot, 1981). Recent discoveries (Teal et al., 2000) have allowed for improved methods of analyzing JH III by MS. These methods are sensitive and allow for quantitative determinations of JH without derivative formation.

In this study, the hemolymph of *S. invicta* female alates is analyzed with GC-MS using chemical ionization with isobutane as the reagent gas.

Materials and Methods

The following techniques for collecting hemolymph, identifying and quantifying natural JH compounds, and analyzing synthetic JH I, II, and III and *trans,trans*-farnesyl acetate are described in Teal et al. (2000).

Chemical Analysis. Gas chromatography-mass spectroscopy analysis was performed with a Finnigan-MAT ITS 40 ion-trap MS operated in chemical ionization (CI) mode and interfaced to a Varian Star 3400 GC. The GC was designed with a CTC Analytics A 200 S autosampler and a cool-on column and split/splitless injectors. The GC was equipped with a DB5-MS capillary column (30m x 0.25mm, i. d.; 0.1um film thickness). With the cool-on-column injector, the analytical column was connected to a 10cm x 0.5mm (i. d.) length of uncoated, deactivated fused silica and a 10m x 0.25mm (i. d.) uncoated, deactivated fused silica retention gap in the injector to permit the introduction of considerable amounts of sample without loss of resolution. The initial injector temperature for chromatography was 40°C for 30s, and temperature increased at 170°C/min to 270°C. The initial column temperature for chromatography was 40°C for 5min, and temperature increased at 5°C/min to 210°C. The He carrier gas linear flow velocity was 24cm/s, and the GC/MS transfer line temperature was 230°C. Under these conditions, farnesyl acetate, JH III, JH II, and JH I eluted at 32.3, 33.8, 35.4, and 37.3min, respectively.

Hemolymph Collection and Analysis. *Solenopsis invicta* monogyne colonies possessing numerous female alates were collected from north central Florida during the spring of 2000.

Crickets, sugar water, and tap water were supplied to each colony. Ants were maintained in the laboratory at 27°C and 47% humidity. For this study, hemolymph from sexually mature *S. invicta* female alates was analyzed. Each alate was pinned dorsally through the thorax at an angle, and pressure was applied with fine forceps to the base of the thorax, allowing hemolymph to emerge from the wound. A fused silica needle (0.15mm, o. d.) held in a 10-ul gas-tight syringe was used to withdraw hemolymph, which was then placed in a conical vial held on ice. Once 5ul of hemolymph was collected (approximately 1ul/alate), the sample was centrifuged at 8000rpm for 2min. This procedure was repeated three times. Forty-five microliters of methanol and 100ul of hexane containing 10pg/ul of farnesyl acetate (internal standard) were added to each hemolymph sample. Crimp caps were placed on vials, and samples were vortexed. Vials were centrifuged at 18000rpm for 5min, and the organic layer was removed. The aqueous layer was extracted an additional two times as described above, and organic layers were combined and analyzed.

Aliquots of alate hemolymph, representing approximately 1.5% of the total organic fraction, were analyzed by GC-MS. Six diagnostic ions for each JH homolog and farnesyl acetate were used for quantitative purposes (Table 6-1).

Further purification of JH was accomplished by Microbore High-Performance Liquid Chromatography (HPLC) (Teal, unpublished data). The organic extracts were pooled and concentrated under N_2 to a volume of 20ul. The sample was injected onto an Adsorbosphere Silica (15cm x 4.6mm, i. d.; 3um particles), with a Rheodyne 7125 injector (50ul loop). The column was eluted at 0.3ml/min with 1% ethanol in hexane, employing a Spectroflow 400 pump. A Spectroflow 757 UV detector set at 210nm was used to detect peaks. Under these conditions, JH III eluted at 4.8min, JH II at 5.5min, and JH I at 6.8min. When the natural extract was chromatographed, fractions were collected from 0-4min, 4-7min (JH fraction), and 7-14min. The fraction containing JH was concentrated to 50ul, and a 3ul aliquot was analyzed by GC-MS.

Results

Analysis of hemolymph from *Solenopsis invicta* female alates revealed the presence of JH III in each of the four 5-ul samples, and the average amount of JH III per microliter of hemolymph was 0.29±0.13pmol. Juvenile hormone III was identified and quantified based on the abundance of ions specific to JH III (Table 6-1). All four hemolymph samples contained m/z=266 (Molecular Ion).

Although JH III was clearly present in crude hexane extracts of hemolymph, the presence of conflicting peaks having GC retention times coincident with JH I and JH II precluded determination of the presence of these homologs. Separation of the organic extract by liquid chromatography and analysis of the JH fraction by GC-MS revealed the presence of only JH III (Figs. 6-2 to 6-3).

Discussion

This study is the first to report the identification and quantitation of JH III in *Solenopsis invicta*. Alates were chosen as subjects for several reasons. First, hemolymph is more plentiful and easier to collect in alates, compared to workers, and mature colonies can possess hundreds of alates. In addition, much attention has been generated about the functions of the queen primer pheromone in controlling dealation (Kearney et al., 1977; Barker, 1978; Vargo and Laurel, 1994; see Chapter III) and ovary development (Barker, 1978; Vargo and Laurel, 1994) in cohabiting female alates, possibly by regulating JH titers.

Juvenile hormone III has been identified as the only JH homolog in a Malaysian *Diacamma* species (Sommer et al., 1993). In this ant species, the queen caste is absent, but an inseminated worker reproduces (gamergate) (Peeters and Crewe, 1984). Unlike

S. invicta, in which the queen pheromone inhibits wing casting of alate nestmates, the Malaysian *Diacamma* gamergate establishes reproductive dominance by physically attacking newly-eclosed workers and removing gemmae, a pair of tiny bladder-like appendages attached to the thorax. Juvenile hormone titers were measured by GC-MS in groups of gamergates and non-reproductive workers to test whether JH influences worker-dominance. No detectable amounts of JH were found in reproductives; however, JH III was identified, and titers were shown to increase with age in non-reproductives. Juvenile hormone levels ranged from 0.9pmol/g in callows of up to 25 days old to 3.6pmol/g in workers of 5 months old to 19.3pmol/g in mature workers of older than 5 months old. The results of Sommer et al. (1993) conflicted with those of JH investigations in many other insects (Koeppe et al., 1985), in that ovarian development was not correlated with JH levels in the *Diacamma* species.

Juvenile hormone III was also identified as the only JH homolog in *Apis mellifera* (Robinson et al., 1987, 1989). This hormone does not appear to be a major factor in vitellogenesis but may regulate age polyethism. Robinson and his colleagues (1991) found that JH titers in mated egg-laying queens, egg-laying workers, and nurses were significantly lower (approximately 5pmol/100ul hemolymph for each group of bees) than those in foragers

(approximately 30pmol/100ul hemolymph). Ecdysteroids were suggested instead as major hormones contributing to ovary development.

Although high JH levels are not associated with reproductive dominance in *A. mellifera* (Robinson et al., 1991) and the Malaysian *Diacamma* species (Sommer et al., 1993), a correlation does exist in many other insects (Koeppe et al., 1985), including *Polistes gallicus*. Röseler et al. (1980) discovered that CA activity in dominant *P. gallicus* females was as high as 3.7pmol JH/CA, while the CA of less dominant females did not produce over 0.7pmol JH/CA.

Results from investigations involving allatectomy and the use of topical applications of JH analogs on *S. invicta* female sexuals have suggested that JH is positively correlated with reproductive dominance (Barker, 1978; Vargo, 1992; Vargo and Laurel, 1994). Barker (1978) reported that allatectomized alates oviposited fewer eggs (39±7 eggs) than alate controls (353±25 eggs); however, 10ug of a synthetic JH, consisting of a mixture of 8 geometric isomers, increased oviposition in allatectomized females (299±33 eggs). Vargo and Laurel (1994) also found that the JH analogue methoprene was very instrumental in increasing ovary development in alates, suggesting that the primer pheromone produced by the

functional queen suppresses JH production. Comparing the amounts of JH titers in both unmated and mated female reproductives would determine whether differences do exist in the levels of JH and might provide additional support to the hypothesis of JH suppression from queen pheromone.

Reproductive dominance of *S. invicta* queens also may involve inhibiting dealation in cohabiting alates. When female alates are not under queen pheromonal influences, alates shed their wings, and wing muscle degeneration takes place. The amino acids and proteins provided by the muscles contribute to the production of eggs (Vinson, 1986). Chapter III demonstrates that topical applications of synthetic JH III, ranging from 0.01-20ng/ul acetone, were sufficient to induce alates to shed their wings while in the presence of the functional queen. Precocene II treatments of 90 and 100ug/ul acetone inhibited dealation in over 80% of alates in queenless colonies, while 95 and 100% of precocene-treated alates shed their wings within 48h of administering 10 and 20ng JH III, respectively. These results, as well as those from other studies (Barker, 1978; Kearney 1977; Vargo and Laurel, 1994), provide evidence of JH's role in wing casting.

The above studies are only a few examples demonstrating the versatility of JH in regulating development and reproduction in some

hymenopteran species. Though JH III is the most predominant of JH compounds, additional JH homologs--JH 0, JH I, JH II, 4-methyl JH I, methyl farnesoate (see Borst and Laufer, 1990 and Baker, 1990 for reviews), and JH III bisepoxide (Rickards and Thomas, 1993; Yin et al., 1995)--may be present in particular stages of insect life. For example, in *Manduca sexta*, JH 0, JH I, JH II, and 4-methyl JH I are present in eggs (Bergot et al., 1981a), and JH I, II, and III function in adults (Schooley et al., 1976).

The regulating capacity of JH has generated much interest in its incorporation in insect pest management strategies that are designed to be environmentally safer than conventional control methods. One of the potential effects of using synthetic JHs in controlling *S. invicta* is that the hormones may encourage premature dealation, thereby reducing the number of alates engaging in mating flights. However, some disadvantages of using JHs as control agents include the instability of the compounds in sunlight (Staal, 1975), the expense of synthesizing large quantities (Nijhout, 1994), and the possibility of insects developing resistance (Hammock, 1985). Nonetheless, the integration of JH or JH antagonists with conventional insecticides may prove beneficial in controlling fire ants.

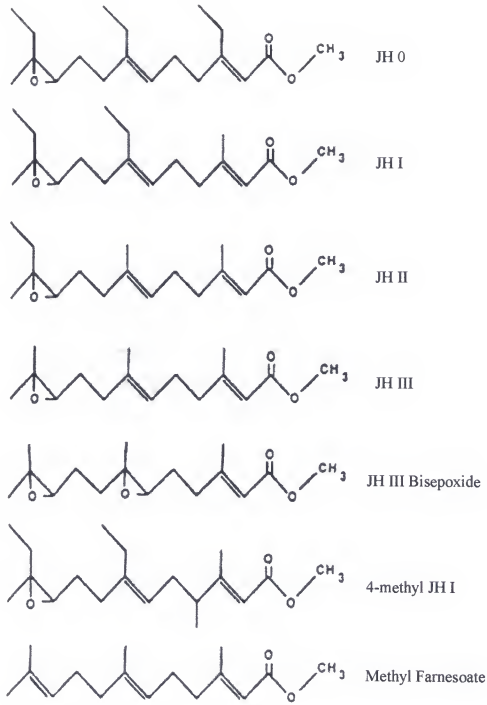

Figure 6-1. Homologs of juvenile hormone (JH).

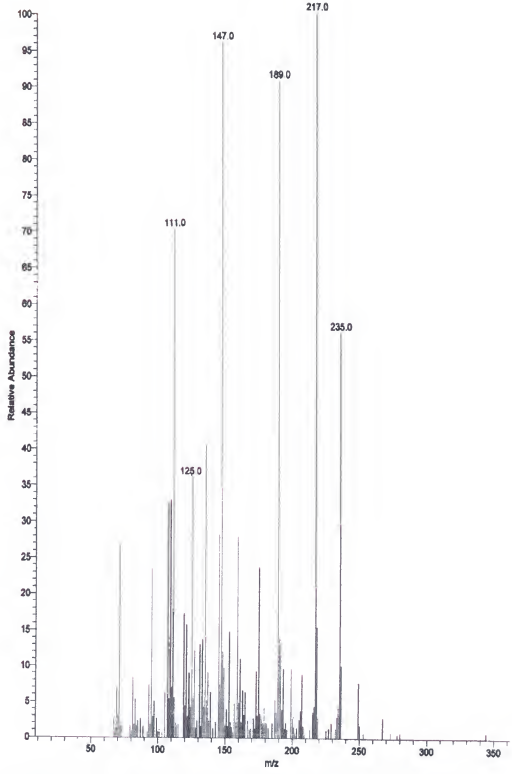

Figure 6-2. CI-MS spectrum obtained from analysis of synthetic JH III.

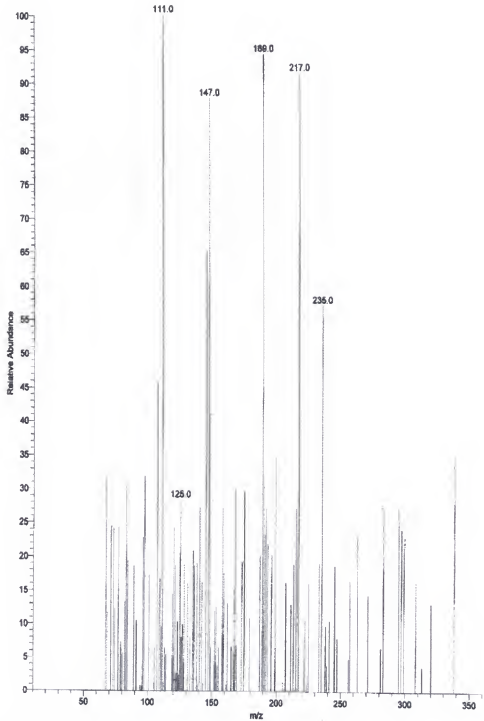

Figure 6-3. CI-MS spectrum obtained from the analysis of naturally produced JH III from the hemolymph of *Solenopsis invicta* female alates.

Table 6-1*. Description of Cleavage Assignments Resulting in Generation of Diagnostic Ions Used for Quantitation of JH I, JH II, and JH III.

	Mass to Charge (m/z)		
Ion Description	JH I	JH II	JH III
M + 1 - CH_3OH (from methyl ester)	263	249	235
Ion 1 - HOH (from ring cleavage of epoxide)	245	231	217
Ion 2 - CO (from methyl ester)	217	208	189
Ion 1 - $C_2H_4O_2$ (from methyl ester) - $C_4H_{10}O$ (from epoxy terminus)	161	147	
Ion 1 - $C_2H_4O_2$ (from methyl ester) - C_3H_8O (from epoxy terminus)			147
M - $C_8H_{13}O_2$ (cleavage between C6 and C7)	153	139	125
$C_4H_{11}O$ (scission between C6 and C7 after loss of CH_3OH	111	111	111

*Taken from Teal et al. (2000)

CHAPTER VII

CONCLUSIONS

Solenopsis invicta female alates normally dealate (shed wings) once they descend from a mating flight; however, dealation can occur within the colony if the functional queen is no longer present. Age/sexual maturity and the presence of workers and brood were two conditions examined to determine whether either influences the time in which alates are stimulated to shed their wings. Results indicated that maturity is not a major factor controlling dealation. However, the exclusion of workers and brood did have an effect on the rates of dealation, in that alates in isolation and those in groups shed their wings later than alates in the presence of workers and brood. Workers were not observed physically assisting alates in wing casting, but tactile and olfactory stimuli or food may be potential factors provided by workers and brood to stimulate dealation.

Topical applications of synthetic juvenile hormone (JH) III and precocene II were administered to sexually mature alates to determine the role of JH in dealation. Applications of JH III, ranging from 0.01-20ng, were sufficient to stimulate alates to shed their

wings while in the presence of the functional queen. The time in which alates were induced to dealate decreased with increasing levels of JH. Precocene treatments of 90 and 100ug inhibited over 80% of alates in queenless colonies from shedding their wings; however, this inhibition was reversed after applying JH to precocene-treated alates.

In addition to examining the time in which alates shed their wings, the sizes of corpora allata (CA) in female alates were measured to determine whether age or JH and precocene applications affect the sizes of the glands. Results indicated that the sizes of CA did not change when female reproductives reach sexual maturity or when alates were treated with 1ng JH III. However, CA sizes were reduced in alates treated with 100ug precocene II. These results, along with those from experiments involving dealation, suggest that JH is the stimulus to wing casting and that the functional queen suppresses natural JH titers in cohabiting alates.

Juvenile hormone appears to be necessary in stimulating female alates to fly. Alates that were topically treated with 100ug precocene II did not display the typical pre-flight behaviors associated with a mating flight. These behaviors consist of alates ascending from the mound and climbing rapidly onto nearby

vegetation. Tethered precocene-treated alates could not be induced to fly.

Along with flight, several other behaviors associated with a mating flight were examined to determine whether one or a combination of behaviors contributes to dealation following the flight. Pre-copulatory behaviors investigated were 1) excited alates scurrying on the surface of the soil, 2) excited alates climbing onto tongue depressors which simulated vegetation, 3) tethered alates stimulated to fly for five minutes but not primed for a mating flight, 4) alates displaying all of the described pre-mating behaviors. The time in which alates exhibiting these pre-copulatory behaviors were induced to shed their wings was longer than that of mated females. Mating, alone or in combination with other behavioral and/or environmental cues, may be a major factor inducing dealation. And because topical JH applications were shown to induce alates to shed their wings earlier than controls, the unknown behaviors and/or environmental conditions contributing to dealation of newly-mated queens may involve an increase of JH levels in these females.

The hemolymph of sexually mature alates was analyzed with gas chromatography-mass spectroscopy and high-performance liquid chromatography to detect naturally-occurring JH compounds.

Results indicated the presence of JH III in these alates, and the average amount of JH III/ul hemolymph was 0.29±0.13pmol. This study is the first to report the identification and quantitation of a JH compound in *S. invicta*. Further analysis of hemolymph from mated females would determine whether JH levels increase after a mating flight, thereby possibly contributing to dealation.

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BIOGRAPHICAL SKETCH

Shuntele N. Burns spent much of her youth in Valley, Alabama, with her paternal grandparents. Following high school, she attended Alabama State University, in Montgomery, where she graduated summa cum laude in biology. Prior to her enrollment in graduate school, she accepted an internship with E. I. du Pont de Nemours and Company in Wilmington, Delaware. During her tenure at the University of Florida, Burns was inducted into Gamma Sigma Delta, the honor society of agriculture, and she received a master's degree in entomology.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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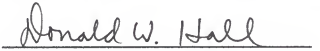

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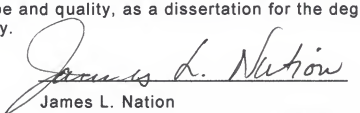

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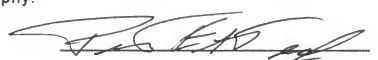

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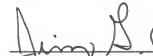
August 2000



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